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Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

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Association of Professors of Medicine
Sixth Congress of the European Society for Medical Oncology

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

COMMENTARY

Phase II Trials: Use of a Clinical Tumor Panel and Overview of Current Resources and Studies [1,2]

Franco M. Muggia,* Marcel Rozencweig, Delia F. Chiuten, Michael S. Jensen-Akula, Lloyd M. Charles, Jr, Thomas T. Kubota, and Vincent H. Bono, Jr [3]

The primary objective of a phase II clinical trial with a new drug is to identify the therapeutic efficacy of the drug in a well-defined population of patients. A qualitative answer regarding the level of antitumor activity and quantitative estimates of the degree of toxicity of the new agent using a specific dose schedule are expected from such studies. Eventually, its therapeutic usefulness will be established further in subsequent comparative studies using standard regimens as concurrent or historical controls, or in studies involving combinations with other agents or other treatment modalities.

Secondary objectives of phase II studies are intimately related to our drug development program. Basically, such trials also provide the feedback needed to evaluate the relevance of current screening systems in selecting new active drugs. The current preclinical screening panel utilizes matched pairs of human breast, colon, and lung xenografts in nude mice and corresponding transplantable mouse tumors; in addition, the L1210 leukemia and B16 melanoma complete this panel. We introduce here the concept of performing studies in a clinical tumor panel to match the preclinical screening panel and we describe current phase II drug studies and resources.

DESCRIPTION OF METHODS AND CLINICAL TUMOR PANEL

In the 1950s and 1960s, trials referred to as "broad phase II trials" were performed to evaluate the activity of a new drug. Such studies included patients with a variety of disseminated tumors, with no consideration given to prognostic factors or the number of patients included for each tumor type. Several drugs received inadequate evaluation as judged by current standards (1). During the past decade, with increasing knowledge of prognostic factors (2) and the influence of statistical concepts (3), phase II trials were transformed into disease-oriented studies. The Division of Cancer Treatment (DCT) introduced the concept of "signal" tumors to indicate those tumor types that should constitute the initial target for phase II studies. To quote Goldin and Carter (3): "This survey is designed to test thoroughly for drug activity against tumors that are kinetically described as 'fast growing' (leukemia, lymphoma) and 'slow growing' (colon, breast, lung)." Subsequently, malignant melanoma was included, presumably because of its frequent dissemination and the ease of evaluating its antitumor effect (4). Later, brain, pancreatic, and ovarian cancers were added to the signal tumor list to broaden the clinical range of phase II studies (5).

More recently, the flow of clinical studies following phase I trials has been restructured to include a required clinical panel of six tumors in phase II studies, while other tumors are optional (fig 1). Major reasons prompting this modification include both the desirability of obtaining data for all new drugs to match the preclinical panel of tumors (ie, murine L1210 and P388 leukemias, colon tumors 26 and 38, Lewis lung carcinoma, B16 melanoma, and the human xenografts including breast, colon, and lung cancer) and the impracticality of testing all new drugs initially in some of the previously designated "signal" tumors. For example, it is unlikely that all new drugs can be tested against brain tumors and pancreatic cancer, which present many difficulties in assessing response. Many conventional agents are yet to be evaluated in these tumor types. It should be repeatedly emphasized, however, that the clinical panel presents a *minimum* requirement for testing new anticancer drugs, and that widespread study in *all* tumor types is to be encouraged, particularly with new chemical agents with unique properties.

Any attempts at correlating the antitumor spectrum of a drug in the preclinical screen with its subsequent performance in phase II studies in the clinical panel must take into account some problems inherent in these cor-

[1]Received Oct 5, 1979; accepted Oct 16, 1979.

[2]Presented at the 8th Joint Working Conference, Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Lancaster, PA, Sept 27–29, 1978.

[3]Cancer Therapy Evaluation Program, DCT, NCI, Bethesda, MD.

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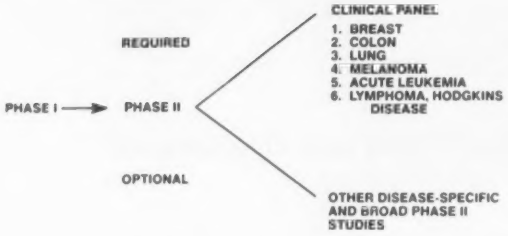

FIGURE 1.—New anticancer drugs: flow of clinical trials.

relations. Even when the clinical studies utilize the same tumor types included in the antitumor screen for identifying new agents, factors inherent in both the tumor and the host render this correlation more complex than one would anticipate. Testing in human tumor xenografts has been introduced because of the appealing concept of utilizing a tumor that retains the characteristics of a human tumor while growing in nude mice. For purposes of screening, only one tumor from each major type was selected. Considering the wide individual variation in responsiveness of tumors of the same histologic type, one must restrict interpretation of the correlation to the *one tumor utilized in the screen*. Experiments utilizing a panel of human tumor xenografts of the same type in nude mice are probably not practical for general screening. However, other techniques, such as growth in subrenal capsule or in soft agar, are being developed which in the future might test the activity of a drug against a spectrum of human tumors of *the same type*.

Another major difference between studies in mice and man is that most patients included in single-agent phase II studies have received extensive prior therapy, a situation that will prevail as initial treatment strategies improve. Thus, one is not strictly comparing the effects of drugs on the same tumor because prior drug exposure is likely to alter many characteristics of both the tumor and the host. In clinical studies, we circumvent this problem to some extent by including a large number of patients from several different institutions, so that populations with variable prior drug treatment are exposed to the new drug. Obvious differences in the host's drug tolerance and immunologic system in the preclinical versus the clinical situation need not be elaborated, but an interesting awareness of immunologic factors leading to tumor regression in nude mice is emerging.

In summary, the clinical panel is a useful operational concept to provide thorough clinical evaluation of drugs emerging from the screen. However, one should not a priori anticipate correlations between drug activity in mice and man with any particular tumor type or with the xenografts as opposed to any autologous mouse tumor.

The methods of phase II trials have been summarized in recent publications (6–8). Particular attention must be given to the following basic items: (*a*) patient characteristics, (*b*) study design, (*c*) definitions and methods of assessing efficacy (response and duration of effects), (*d*) definitions and methods of assessing toxicity, and (*e*) format for reporting results. Features of patient selection applicable to the clinical panel need elaboration. The number of patients to be included in studies of each disease category will vary according to the importance attributed to specific prognostic variables in determining antitumor responses (6). Separate trials are needed for the various histologic types of lung cancer since the response rates to a chemotherapeutic agent or agents may vary, especially when one contrasts small cell carcinomas with other cell types (9). In addition, one might consider separate analyses for patients with cutaneous versus other sites of breast cancer metastases or with cutaneous versus visceral sites of involvement in malignant melanoma (6). Finally, testing of drugs in leukemia and lymphoma has become quite complex due to the wide range of effective therapies that must precede exposure to new drugs and because of the various subtypes of each disease category. However, since these malignancies are generally responsive to a wide variety of cytotoxic drugs, it is possible that preliminary activity can be detected in relatively small trials.

In order to correlate data reported by different investigators, there should be agreement on standardizing definitions, methods, and data-reporting. Areas which require special attention are the timing of the objective measurement to qualify as a partial regression, the endpoints in measuring response duration, the application of rules when multiple indicator lesions are present, and the various definitions of treatment failure. Although investigators often report less than partial or minor regressions, so far these responses appear to be of doubtful significance. Also, a new drug should be exclusively evaluated in patients where it is possible to obtain measurable objective changes. In conditions where measurable disease is a rare occurrence, every effort should be made to enter preferentially such patients into phase II trials of new drugs or new drug combinations whenever indicated.

The study design most commonly employed is one that enters consecutive patients with a given tumor type into a trial of an experimental drug at a given dose schedule. Statistical considerations define the minimum number of patients to be studied (8,10)[4] and, once responses are established, there are techniques which outline the entry of additional patients (11). More complex study designs may yield additional information and may be employed if circumstances permit. With the availability of two new drugs or drug regimens for phase II testing, it might be

[4]Report by E. A. Gehan. Presented at the 8th Joint Working Conference, DCT, NCI, Lancaster, PA, Sept 27–29, 1978.

advantageous to randomize between these two treatments (6). This not only eliminates entry bias but also provides a greater chance for patients to be exposed to an active regimen. In addition, information on crossover from one drug regimen to another may also be obtained.

RESOURCES FOR PHASE II STUDIES

A phase I–II working group has been established, which includes large individual institutions committed to new drug testing and supported partly by contracts (table 1). Representatives of grant-supported cooperative groups, usually chairmen of new agents committees, and ad hoc members representing either unique expertise (such as in pediatrics) or interest in a particular drug are also included. Phase II trials are initiated once phase I studies are completed for at least one schedule and reviewed in the presence of the working group and by the Decision Network committee. The step referred to as Decision Network IV indicates concurrence with the finding of a dose schedule which is suitable for more widespread testing in phase II trials.

Figure 2 illustrates the extent and relationship of study resources for phase II studies. Protocols for phase II studies including the clinical tumor panel are initiated by the phase I–II working group. Experience with cancer treatment and the availability of large numbers of patients suitable for study add to the reliability of studies within these institutions. Thorough documentation of relevant prognostic variables, prior treatment exposure, and methods to assess response are provided in each instance. Other studies may be carried out by the grant-supported cooperative groups (table 2) or by contract-supported study groups (table 3); many of these groups have special expertise in certain disease areas. Phase II studies in lymphoma and pancreatic, gastric, gynecologic,

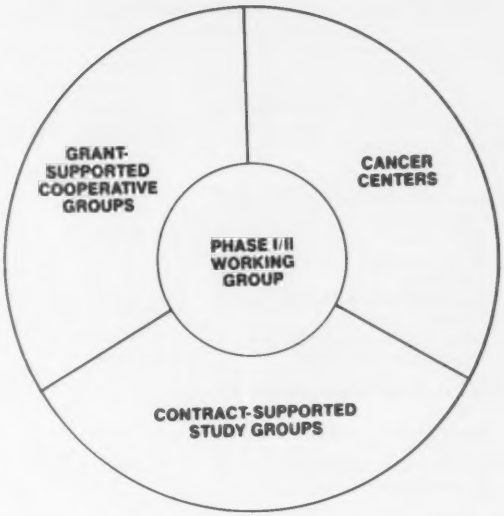

FIGURE 2.—New anticancer drugs: study resources.

TABLE 1.—Phase I-II Working Group

Institute	Principal investigator
Baltimore Cancer Research Program, DCT, NCI	P. Wiernik
Children's Hospital of Los Angeles	S. Siegel
Clinical Pharmacology Branch, Clinical Oncology Program (COP), DCT, NCI	B. Chabner
Georgetown University	P. Woolley*
Mayo Clinic	J. Kovach*
M. D. Anderson Hospital and Tumor Institute	G. Bodey*
Medicine Branch, COP, DCT, NCI	R. Young
Memorial Sloan-Kettering Cancer Center	C. Young*
Mt. Sinai Cancer Center	T. Ohnuma*
NCI-Veterans' Administration Medical Oncology Branch, COP, DCT, NCI	J. Minna
Ohio State University	J. Neidhart
Pediatric Oncology Branch, COP, DCT, NCI	A. Levine
Roger Williams General Hospital	F. Cummings
Roswell Park Memorial Institute	A. Mittelman
Sidney Farber Cancer Institute	E. Frei*
St. Jude Children's Research Hospital	C. Pratt
University of California at Los Angeles	J. Block
University of Kansas	B. Hoogstraten*
University of Miami	C. Vogel
University of Vermont	I. Krakoff*
University of Wisconsin	H. Davis*
Wayne State University	L. Baker*
Yale University	J. Bertino

*Contract-supported.

TABLE 2.—Grant-supported groups involved in phase II trials

Group	Chairman
Cancer and Leukemia Group B (CALGB)	J. Holland
Children's Cancer Study Group (CCSG)	D. Hammond
Eastern Cooperative Oncology Group (ECOG)	P. Carbone
European Organization for Research on Treatment of Cancer (EORTC)	H. Tagnon
Gynecologic Oncology Group (GOG)	G. Lewis
Northern California Oncology Group (NCOG)	S. Carter
Radiation Therapy Oncology Group (RTOG)	S. Kramer
Southeastern Cancer Study Group (SEG)	J. Durant
Southwest Oncology Group (SWOG)	B. Hoogstraten

TABLE 3.—Other contract-supported activities involved in phase II trials

Activity and institution	Chairman (*) or principal investigator
Brain Tumor Study Group 14 institutions	M. Walker*
Phase II–III studies in disseminated solid tumors	
Mayo Clinic	J. Edmonson
M. D. Anderson Hospital and Tumor Institute	G. Bodey
Memorial Sloan-Kettering Cancer Center	J. Burchenal
Sidney Farber Cancer Institute	G. Canellos
Wayne State University	L. Baker
Phase II studies in gastrointestinal cancer	
Georgetown University	P. Schein
Gastrointestinal Tumor Study Group 20 institutions	C. Moertel* and P. Schein*
Miscellaneous	
National Cancer Institute, Milan, Italy	U. Veronesi and G. Bonadonna
World Health Organization (melanoma)	U. Veronesi

and pediatric cancer are being extensively conducted by cooperative groups, often utilizing multiple-arm designs. These may include testing combinations as well as single agents through randomization, or testing new agents following their primary protocols in a shifting priority sequence (ie, one new agent being first in line for a certain number of patients, and then shifting places with the agent second in line). Finally, if additional studies appear warranted, drugs are also made available to investigators in cancer centers under the aegis of the center's Director or his designee (table 4). This provision has been introduced in order to take advantage of original ideas within cancer centers having investigators with special expertise in a drug that would otherwise not be available to them.

A great deal of duplication of phase II studies may occur among the various phase II study resources. This might be desirable in order to adequately evaluate the clinical tumor panel. As stated earlier, the rationale for duplicate efforts derives in part from the unpredictable effect of prior therapy in modulating response rates. Thus, studies conducted in a number of institutions, where prognostic features of the patient population might differ, could yield divergent results. Moreover, additional observations on toxicity might result from the experience of various investigators with one agent. In fact, important observations have often been made by one investigator who introduced a new method for monitoring toxicity that was neglected or not observed by others. Of even greater significance are efforts in modifying toxic reactions which become apparent from specific studies. An obvious example is the circumvention of *cis*-dichlorodiammineplatinum(II) nephrotoxicity by vigorous hydration and monitoring of diuresis. Of course, the degree to which duplication is desirable will vary with the disease in question, the extent to which conflicting preliminary results have been obtained, and the quality and originality of new studies vis-a-vis older ones. Further trials in a disease such as breast cancer might be legitimately discouraged if the drug has been ineffective in one properly designed trial. However, a second trial would be warranted if it was directed toward a selected population of patients more likely to respond, and certainly if a newer mode of administration was introduced.

The availability of several studies in a single disease also permits the exploration of more than one dose schedule. The extent to which schedule alterations should be studied is another uncertain aspect, and one that is likely to vary with the type of agent. For example, the activity of cytosine arabinoside in acute leukemia was not adequately recognized until continuous-infusion schedules were utilized (12). 6-Diazo-5-oxo-L-norleucine (DON) is another drug whose tolerance is markedly time-dependent (13). Its reintroduction into clinical trial has been prompted by documentation of its activity against human tumor xenografts in the preclinical screening panel (14) and by its previously incomplete evaluation, even in the single daily dose schedule which would be expected to be most toxic to the gastrointestinal tract. It will be important to explore not only the daily schedules used in the 1950s, but a variety of new schedules including high intermittent doses. It is also conceivable that the efficacy of a drug may vary with schedule in one tumor type but not in another.

A major advantage in the multiplicity of phase II study resources is the ability to launch studies employing a new drug within a short period of time, while interest is high and information on it has received widespread recent attention. Having many simultaneous studies also ensures more statistically reliable conclusions, provided that they are based on analysis of results obtained at the same dose schedule and in the same target patient population. Finally, publication of the findings in a well-defined reporting format (6,8) will avoid any ambiguities which may otherwise arise.

CURRENT STUDIES

Anticancer drugs introduced into clinical trials by the DCT in the past 3 years are shown in table 5. These have been subdivided into synthetic drugs, natural products, and analogs. The table does not include the biologic agents bacillus Calmette-Guérin and *Corynebacterium*

TABLE 4.—Participating cancer centers

State	Cancer center	Director
Alabama	University of Alabama Comprehensive Cancer Center	J. R. Durant
Arizona	University of Arizona Cancer Center	S. Salmon
California	University of California at Los Angeles Comprehensive Cancer Center	R. J. Steckel
California	Northern California Cancer Program	S. K. Carter
California	Stanford University Medical Center	H. S. Kaplan
California	University of California, San Diego	J. Mendelsohn
Colorado	Colorado Regional Cancer Center, Inc	S. G. Silverberg
Connecticut	Yale University Comprehensive Cancer Center	J. W. Cole
Washington, DC	Vincent T. Lombardi Cancer Research Center	J. F. Potter
Washington, DC	Howard University College of Medicine	J. E. White
Florida	Comprehensive Cancer Center for the State of Florida	C. G. Zubrod
Georgia	Emory University Cancer Center	C. M. Huguley, Jr
Hawaii	University of Hawaii	L. H. Piette
Illinois	University of Chicago Research Center	J. E. Ultmann
Illinois	Rush-Presbyterian-St. Luke's Medical Center	F. R. Hendrickson
Illinois	Northwestern University	N. I. Berlin
Maryland	Johns Hopkins Oncology Center	A. H. Owens, Jr
Massachusetts	Sidney Farber Comprehensive Cancer Center	E. Frei III
Massachusetts	Boston University Cancer Research Center	S. Cooperband
Minnesota	Mayo Comprehensive Cancer Center	C. G. Moertel
Missouri	Missouri Cancer Programs, Inc	J. W. Yarbro
New Mexico	Cancer Research and Treatment Center	M. M. Kligerman
New York	Sloan-Kettering Institute for Cancer Research	R. A. Good
New York	Roswell Park Memorial Institute	G. P. Murphy
New York	Cancer Research Center	H. Eagle
New York	University of Rochester Cancer Center	R. A. Cooper, Jr
North Carolina	Duke University Comprehensive Cancer Center	W. W. Shingleton
North Carolina	Oncology Research Center, Bowman Gray	Charles L. Spurr
Ohio	The Ohio State University Comprehensive Cancer Center	D. S. Yohn
Ohio	The Cancer Center, Inc	A. Flynn
Oklahoma	Oklahoma Cancer Center	G. B. Humphrey
Pennsylvania	Fox Chase Cancer Center	T. R. Talbot, Jr
Pennsylvania	University of Pennsylvania Cancer Center	R. A. Cooper
Rhode Island	Roger Williams General Hospital	P. Calabresi
Tennessee	Memphis Regional Cancer Center	J. J. Nickson
Tennessee	St. Jude Children's Research Hospital	A. M. Mauer
Texas	The University of Texas System Cancer Center	R. L. Clark
Texas	University of Texas Health Science Center	E. P. Frenkel
Vermont	Vermont Regional Cancer Center	I. Krakoff
Washington	Fred Hutchinson Cancer Research Center	W. B. Hutchinson
Wisconsin	University of Wisconsin Clinical Cancer Center	H. P. Rusch
Wisconsin	Medical College of Wisconsin	D. P. Pinkel

parvum or the diuretic spironolactone, which was included in certain trials of gastrointestinal malignancies. Table 6 illustrates studies being carried out with a representative drug from each of these categories.

The synthetics include new cytotoxic drugs (exclusive of the analogs PALA [*N*-(phosphonacetyl)-L-aspartic acid], pyrazolo imidazole [2,3-dihydro-1*H*-pyrazolo[2,3-*a*]imidazole; IMPY], and 3-deazauridine), some known drugs introduced to determine their antitumor activity (thymidine, 2-amino-1,3,4-thiadiazole, and hycanthone), and some classes of compounds representing new areas of investigation (misonidazole and levamisole). With new cytotoxic drugs, the plan of clinical investigation includes rapid introduction into phase II studies, as is shown for AMSA [4′-(9-acridinylamino)methanesulfon-*m*-anisidide] (table 6). This drug was introduced in 1977; phase I testing was completed in 1978 (15) and a number of phase II studies in the clinical panel and in other diseases are being completed. Generally, two schedules have been used for this myelosuppressive drug, ie, daily × 3 days and a single dose, both repeated every 3 weeks. With a nonmyelosuppressive drug such as PALA, which is completing phase I studies, at least two divergent dose schedules are likely to be tested in phase II studies in an effort to circumvent or render predictable the skin and gastrointestinal toxicity of this agent.

TABLE 5.—Anticancer drugs introduced into clinical trials by the DCT, NCI (1975–1978)

Synthetics	Natural products	Analogs
Thymidine	L-Alanosine	DON*
PALA	Indicine-*N*-oxide	Dichloroallyl lawsone
AMSA	Bruceantin	PCNU
Misonidazole	Maytansine	Pentamethylmelamine
IMPY	Pyrazofurin	ICRF-187
3-Deazauridine	Anguidine	Rubidazone
Levamisole		Chlorozotocin
2-Amino-1,3,4-thiadiazole		AD-32*
Hycanthone		

*Studies initiated at specific institutions partly with DCT support: DON (Memorial Sloan-Kettering Cancer Center); AD-32 (Sidney Farber Cancer Institute).

Thymidine represents an interesting new wrinkle in drug evaluation. Its clinical testing was prompted by activity against a spectrum of human tumor xenografts when sustained millimolar amounts were delivered to nude tumor-bearing mice (16). The safety of duplicating these circumstances in man was demonstrated by Giovanella upon himself. Phase II studies began immediately, and mimicked the same schedule and pharmacologic circumstances which were successful against the xenografts, thus requiring careful monitoring of blood levels as the endpoint for drug scheduling.

The introduction of misonidazole represents still another facet of drug evaluation. It is one of many hypoxic cell radiosensitizers being developed through a "linear array" process different from that of the conventional chemotherapeutic agents. A compound such as levamisole, which was introduced as an immunostimulating agent, will also require entirely new "logic" systems for selecting drugs for clinical trials. The clinical evaluation required for these compounds will probably differ substantially from that used for the other anticancer drugs. It may be desirable to proceed swiftly into comparative trials and combination studies in order to demonstrate

TABLE 6.—Phase II protocol studies in the clinical tumor panel with 3 representative drugs

Tumor	Protocol studies*		
	AMSA	Maytansine	Chlorozotocin
Breast	MDA WSU	MDA Mayo Clinic SWOG WSU	WSU Mayo Clinic
Colorectal	MDA WSU GITSG	Mayo Clinic	GITSG WSU Leeds Mayo Clinic GU
Lung (non-small cell)	WSU MDA	BCRP Mayo Clinic	Mayo Clinic SWOG
Lung (small cell)	WSU MDA	ECOG Mayo Clinic	ECOG SWOG
Melanoma	ECOG MDA	Mayo Clinic WSU MDA	Mayo Clinic ECOG WSU SEG
Acute lymphocytic leukemia	BCRP MDA	SWOG	†
Acute myelocytic leukemia	BCRP MDA	SWOG	†
Lymphoma	MDA	ECOG SWOG	WSU †

*MDA = M. D. Anderson Hospital and Tumor Institute; WSU = Wayne State University; GITSG = Gastrointestinal Tumor Study Group; Leeds = University of Leeds; GU = Georgetown University; BCRP = Baltimore Cancer Research Program. For others *see* table 2.

†Also being tested through international agreements and broad phase II studies.

convincingly both drug efficacy and the importance of the biologic principle that is being tested.

Recognition of the special problems associated with isolating and identifying natural products with anticancer activity has led to an increasing representation of these new structures among drugs advanced for clinical trials (17). Studies with maytansine were recently completed (18) (table 6). Unfortunately, the cross-resistance between this mitotic inhibitor and the vinca alkaloids will complicate interpretation of the largely negative experience achieved to date. Anguidine and pyrazofurin have also received extensive phase II testing, whereas L-alanosine, indicine-*N*-oxide, and bruceantin are completing phase I study. Well-defined mechanisms of action, such as inhibition of orotidylic decarboxylase by pyrazofurin, have led to exploration of combination schemes based on biochemical rationales (19) in spite of the lack of significant antitumor activity in phase II studies.

Analogs represent the most rapidly expanding group of drugs being introduced into clinical trials. Second-generation anticancer drugs are introduced for purposes of enhancing therapeutic index or expanding antitumor spectrum, or for some special pharmacologic rationales (20). In the anthracycline area, in particular, drugs with diminished cardiotoxicity have been actively sought (21) and have, in part, resulted in the introduction of rubidazone and AD-32; more analogs will soon be forthcoming for clinical study. Chlorozotocin is also an example of a drug introduced to bypass troublesome toxicity, in this instance the marrow depression associated with haloethyl nitrosoureas (22). Completion of the clinical panel testing has been accomplished (table 6) and some indication of partial attenuation of toxicity, with retention of antitumor effects, has been noted. This will undoubtedly facilitate incorporation of the drug into combinations and subsequent comparative trials. Enhanced therapeutic indices are also expected from trials with new dose schedules of the glutamine antagonist DON or with a new derivative of lapachol, dichloroallyl lawsone.

The introduction of pentamethylmelamine (23) and ICRF-187 (24) represents development of a parenteral form of a compound when oral forms indicate some antitumor activity but reliability of drug intake or absorption is in question. In the case of PCNU, the drug was targeted for the treatment of brain tumors because its partition coefficient was judged to be optimal among the nitrosoureas for central nervous system penetration. These analogs are currently in phase I trials. The type of phase II evaluation which is carried out with any one analog is probably influenced by the rationale for introduction of the compound, and by its relationship and prior experience with the parent compound. However, because there is a variable relationship among analogs (21), full testing in the clinical panel is also recommended for these drugs.

CONCLUSIONS

The DCT has emphasized drug development in order to introduce new methods for treating currently incurable malignant diseases. The therapeutic successes recently achieved with chemotherapy in some advanced cancers, such as Hodgkin's disease (25), testicular cancer (26), and ovarian cancer (27), provide strong support for such an approach. In this overall strategy, the initial search for antitumor efficacy of a new drug takes place in phase II trials. These disease-oriented studies provide the basis for identifying new therapies that might prove effective against one or more of the vast array of human malignancies (28).

Although the objectives of phase II studies are quite clear, major problems exist when considering the biologic features of the disease under study, the statistical validity of the conclusions, and ethical decisions. Most patients entered in these trials have received extensive prior therapy, and the biologic features of their tumors may be quite different from those in previously untreated patients with lower tumor burdens. If at all possible, it is important to carefully select those patients having diseases with a favorable prognosis that can be easily assessed for response. Statistical considerations have been discussed elsewhere;[5] we have indicated here some of the reasons for extensive testing by several schedules and in a variety of institutions. Adequate resources to carry out these studies have been developed so that thorough and reliable clinical data can be obtained with each drug that has met the requirements for clinical development.

A common ethical question is whether treatments with little chance of curative potential are justified. However, the alternative prospects for these patients include withholding anticancer treatment in the face of progressive illness or applying other treatments with limited usefulness or for which information is equally lacking (29). Another related ethical problem is whether new drug testing can be accomplished *prior* to conventional therapy. Although no direct answer is possible, such testing generally becomes no longer feasible whenever the "conventional" therapy has been shown unequivocally to be effective as defined by cures or prolongation in survival.

Finally, one must view phase II studies in the context of the "screening" experiment. Antitumor activity of new agents has been traditionally detected or confirmed in animal screening systems before introduction of the drugs into clinical trials. Recently, these systems have been revised (30)[6] by introducing a disease-specific orientation utilizing a panel of spontaneous and allografted murine tumors as well as human tumor xenografts. The

[5] *See* footnote 4.

[6] Report by J. M. Venditti. Presented at the 8th Joint Working Conference, DCT, NCI, Lancaster, PA, Sept 27–29, 1978.

practical implications of this screening remain to be determined, and this question formed the basis for the 8th Joint Working Conference held in Lancaster, PA, in September 1978.

Proper clinical feedback will be necessary for assessing the efficacy of the preclinical panel in identifying new active structures. In this context, the clinical panel and the resources to perform phase II studies, which were described, constitute an important feature of drug development. Thus, incomplete or meaningless investigations (31) may be avoided. By contrast, currently introduced drugs receive greater and more systematic evaluation, including as a *minimum* the tumors indicated in the clinical panel studied by standard methods of evaluation. Results obtained in this manner can then be correlated with the drug's antitumor spectrum in the preclinical panel. One assessment of the efficiency of the screening system may be obtained by considering the activity in each of the clinical panel categories and recording the number of positive or negative answers for each drug.

With progress in the development of new practical methods for detection of antitumor efficacy of drugs, it is likely that modifications of the current screen will be introduced. Therefore, a baseline measurement of the efficacy of the current system will be an important determination for the future. This logic for the development and testing of new cytotoxic drugs is applicable, with modifications, to the evaluation of radiosensitizers and biologic response modifiers that may have applications in cancer treatment. When initiating any new long-range efforts, it is important to maintain continuity and feedback between the development of these systems and the results obtained from testing their products.

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High-Dose Methotrexate Used Alone and in Combination for Measurable Primary or Metastatic Osteosarcoma [1,2,3]

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SUMMARY

High-dose methotrexate with leucovorin rescue was used alone or in combination with Adriamycin and cyclophosphamide for the treatment of 27 osteosarcoma patients with measurable indicators of disease. Three patients developed complete responses of measurable lesions, two had partial responses, two had static disease, one had symptomatic improvement, and one had return to normal of physical findings following treatment of a flat bone primary osteosarcoma. While the doses and frequency of administration of high-dose methotrexate differed from those used by previous investigators, these results suggest that aggressive treatment with high-dose methotrexate must be attempted to further evaluate its efficacy as single-agent therapy for osteosarcoma patients not eligible for adjuvant chemotherapy trials.

[Cancer Treat Rep 64:11–20, 1980]

Following the demonstration of the therapeutic effect of high-dose methotrexate (HDMTX) for the treatment of pulmonary metastases of osteosarcoma (1–4), this newer method of administration of one of the original antimetabolites has been adopted for single-agent adjuvant therapy and for multiple-agent adjuvant schemes for the prevention of pulmonary metastases (5–9). For patients with "classic" osteosarcoma, the role of HDMTX and its merits have been accepted promptly, without assessment of its value through phase III clinical trials, as has been the usual practice for the evaluation of most new agents that have reached wide-spread application and acceptance.

Reports on the value of HDMTX in the treatment of measurable primary or metastatic osteosarcoma have come almost exclusively from a few institutions (1,2, 10,11). The purpose of this paper is to review the therapeutic effects of HDMTX used alone and in combination for our patients with measurable lesions of primary or metastatic osteosarcoma. Additionally, the effect of HDMTX used alone and in combination was examined following the removal of pulmonary metastases which developed during previous adjuvant chemotherapy including HDMTX.

PATIENTS AND METHODS

High-Dose Methotrexate-Leucovorin

Between March 1972 and July 1979, 22 patients with osteosarcoma received treatment with increasing doses of HDMTX followed by leucovorin rescue (table 1). MTX was delivered in a 6-hour infusion and was followed by leucovorin rescue at a dose of approximately 5% of the total dose of MTX delivered. Sixteen of these 22 patients had measurable indicators of disease activity. Six patients who developed pulmonary metastases while receiving adjuvant Adriamycin-cyclophosphamide-HDMTX were treated with increasing doses of HDMTX following removal of pulmonary metastases (table 2). Details regarding doses of MTX, numbers of treatments, and intervals between treatments for each of the patients are discussed in the Results section.

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TABLE 1.—HDMTX for osteosarcoma patients with measurable indicators of disease

Clinical status at time of treatment	Patient No.	Disease involvement	No. of MTX infusions, dose	Response after chemotherapy
Primary unresectable tumor of flat bone	1		3, 1250–5000 mg/m²	None
	2		3, 5000–10,000 mg/m²	None
	3		4, 5000–15,000 mg/m²	None
	4		4, 5000–15,000 mg/m²	None
Pulmonary and bony metastases at diagnosis, primary in femur, not resected	5		8, 2500–12,500 mg/m²	Partial, pulmonary metastases only (7 mos)
Pulmonary metastases at diagnosis	6	(Chondrosarcoma)	7, 2500–7500 mg/m²	Partial (7 mos)
	7		4, 5000–15,000 mg/m²	None
Pulmonary metastases following amputation	8		6, 2500–15,625 mg/m²	Complete (3 wks), then additional metastases
	9		4, 2500–10,000 mg/m²	None
	10		7, 2500–10,000 mg/m²	Decrease in pleural effusion
Pulmonary metastases (not resected) developing during adjuvant Adriamycin-cyclophosphamide-HDMTX	11		1, 5000 mg/m²	None
	12		3, 5000–7500 mg/m²	None

TABLE 1.—HDMTX for osteosarcoma patients with measurable indicators of disease—Continued

Clinical status at time of treatment	Patient No.	Disease involvement	No. of MTX infusions, dose	Response after chemotherapy
	13		5, 5000 mg/m²	None
	14		1, 5000 mg/m²	None
Pulmonary and bony metastases following earlier adjuvant Adriamycin-cyclophosphamide-HDMTX	15		3, 5000 mg/m²	None
	16	+ mandible	2, 5000 mg/m²	None

Adriamycin-Cyclophosphamide-High-Dose Methotrexate-Leucovorin

Fifteen patients received Adriamycin (25 mg/m²) for 2 consecutive days followed by cyclophosphamide (600 mg/m²) on the day after the second dose of Adriamycin (table 3). HDMTX, generally in a dose of either 2500 mg or 5000 mg/m², was administered approximately 2 weeks after treatment with Adriamycin and cyclophosphamide. Leucovorin rescue consisted of the administration of 125 mg/m² of calcium leucovorin given in eight divided doses at 3-hour intervals, beginning 3 hours following the completion of the MTX infusion. Leucovorin rescue was continued for 72 hours in most of these patients, with the administration of 12 mg/m² im at 6-hour intervals for the last 48 hours. Thirteen patients had measurable indicators of disease activity.

RESULTS

High-Dose Methotrexate-Leucovorin

Results of treatment of these patients are presented in table 1, categorized by the clinical setting at the time of treatment.

One patient (No. 1) received increasing doses of MTX (1250, 2500, and 5000 mg/m² at 2-week intervals) for unresectable primary osteosarcoma of the frontal bone. Leucovorin rescue was administered over 24 hours to this patient in doses that were approximately 5% of the MTX dose. There was no evidence of response after these treatments, which were the first treatments given at this hospital (in 1972) using HDMTX for osteosarcoma. Another patient (No. 2) with an unresectable primary osteosarcoma arising from the transverse process of the third lumbar vertebra more recently received MTX (5000, 7500, and 10,000 mg/m² at 10-day intervals); there was rapid growth of tumor during the MTX treatment period, and for this reason other chemotherapy was initiated. Two additional patients (Nos. 3 and 4) with osteosarcoma of the ilium failed to respond following weekly treatment with MTX (5000–15,000 mg/m² at weekly intervals).

Among the patients with pulmonary metastases at diagnosis, an 11-year-old boy (patient 5) with a primary tumor of the distal femur with multiple pulmonary, spine, and skull metastases received increasing doses of MTX (from 2500 to 12,500 mg/m²). There was evidence of partial response of the pulmonary metastases following these treatments given at weekly intervals. An additional patient (No. 6), a 7-year-old boy with extraosseous chondrosarcoma located below the knee, was treated following amputation. He received increasing doses of MTX (from 2500 to 7500 mg/m²) with leucovorin rescue. There was evidence of partial response to this therapy with a decrease in the size as well as the number of all pulmonary metastases before the addition of Adriamycin and cyclophosphamide. With the combination of these agents, partial response continued for 7 months.

For four patients (Nos. 7–10) who received MTX following the development of pulmonary metastases, treatment consisted of MTX infusions of 2500–15,625 mg/m² administered at 1–2-week intervals. One patient (No. 8) had complete disappearance of two metastatic lesions; this response persisted for only 3 weeks and was then followed by the appearance of additional metastases. One patient (No. 9) failed to respond. Another patient (No. 10), previously treated with Adriamycin-cyclophospha-

TABLE 2.—HDMTX for osteosarcoma patients after resection of pulmonary metastases detected during adjuvant Adriamycin-cyclophosphamide-HDMTX: increased doses and frequency of MTX infusions

Patient No.	Pre-resection clinical status	No. of MTX infusions	Dose	Response after chemotherapy
17		12	5000 mg/m²	No recurrence, 48+ mos
18		7	5000–7500 mg/m²	Recurrence, 10 mos
19		8	5000 mg/m²	Recurrence, 6 mos
20		9	2500–7500 mg/m²	Recurrence, 2 mos
21		7	5000 mg/m²	Recurrence, 5 mos
22		5	2500–6250 mg/m²	Recurrence, 1 mo

mide adjuvant chemotherapy, had a decrease in his pleural effusion; this decrease lasted for > 6 weeks.

Four patients (Nos. 11–14) who developed pulmonary metastases while receiving adjuvant Adriamycin-cyclophosphamide-HDMTX (2500 mg/m²) (10) subsequently received MTX at doses of 5000–7500 mg/m² at 1–2-week intervals; none of these patients responded following these treatments. Two of the four patients (Nos. 11 and 14) had only one treatment with the increased dose of MTX before refusing additional therapy.

Two patients (Nos. 15 and 16) who developed pulmonary and bony metastases following completion of adjuvant Adriamycin-cyclophosphamide-HDMTX therapy failed to respond after similar escalation of the HDMTX dose or more frequent administration of the agent (10).

Six patients (Nos. 17–22) who had pulmonary metastases of osteosarcoma detected during treatment with adjuvant Adriamycin-cyclophosphamide-HDMTX were treated with MTX (2500–7500 mg/m² at 1–3-week intervals) following resection of their unilateral pulmonary metastases (table 2). Five patients (Nos. 18–22) had single metastatic lesions, and one patient (No. 17) had five metastases involving one lung. Five of these six patients developed recurrent pulmonary metastases at 1–10 months following resection of the original metastatic lesion. Only patient 17, who had five metastases resected from the left lung, remains free of evidence of tumor 4 years following resection of metastases. This patient received 5000 mg/m² of HDMTX at 3–4-week intervals for 1 year in conjunction with monthly injections of an autologous osteosarcoma tumor cell vaccine, which was prepared from his primary tumor, grown in tissue culture, infected with A/Port Chalmers and B/Michigan influenza virus, and inactivated with formalin prior to injections (12).

Adriamycin-Cyclophosphamide-High-Dose Methotrexate

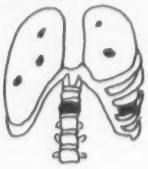
Results of treatment of patients with combination chemotherapy for specific clinical indications are shown in table 3. The numbers of treatments with Adriamycin-cyclophosphamide-HDMTX and the doses of MTX are shown.

For four patients (Nos. 23–26) with primary unresectable tumor of the flat bone (10) following biopsy or partial or incomplete resection, treatments consisted of Adriamycin-cyclophosphamide-HDMTX; radiation therapy was not administered to these patients. A 4-year-old boy (patient 23) with incompletely resected orbital osteosarcoma was treated for 7 months, with a return to normal of all physical findings before he developed Adriamycin cardiotoxicity. He received a cumulative Adriamycin dose of 483 mg/m² over a period of 6 months (13,14). Autopsy findings were consistent with the histopathologic features of Adriamycin cardiomyopathy, and a single focus of tumor was found in the supraorbital ridge.

TABLE 3.—Adriamycin-cyclophosphamide-HDMTX for osteosarcoma patients with measurable indicators of disease

Clinical status at time of treatment	Patient No.	Disease involvement	No. of combination treatments	HDMTX doses	Response after chemotherapy
Primary unresectable tumor of flat bone	23	Orbit, incomplete resection	6	2500, 5000 mg/m²	Return to normal of physical findings before he died of Adriamycin cardiotoxicity (483 mg/m²); single focus of tumor found at autopsy
	24	Scapula, biopsy only	1	2500 mg/m²	None
	25	Zygoma (11 yrs after radiation therapy for retinoblastoma)	3	2500, 5000 mg/m²	None
	26	Clavicle, incomplete resection (8 yrs after radiation therapy for Hodgkin's disease)	10	2500 mg/m²	Complete (3 yrs, 4 mos)
Pulmonary metastases at diagnosis	27		4	5000–10,000 mg/m²	None
	28		4	2500, 5000 mg/m²	None
	29		5	2500 mg/m²	None
	30		3	2500, 5000 mg/m²	None
	31		4	2500, 5000 mg/m²	Complete (5 mos)
Primary and nodal metastases at diagnosis	32	(Down's Syndrome)	2	5000 mg/m²	None, fatal MTX toxicity
Pulmonary and nodal metastases following diagnosis (no adjuvant chemotherapy)	33		5	2500 mg/m²	Partial (5 mos)

TABLE 3.—Adriamycin-cyclophosphamide-HDMTX for osteosarcoma patients with measurable indicators of disease—Continued

Clinical status at time of treatment	Patient No.	Disease involvement	No. of combination treatments	HDMTX doses	Response after chemotherapy
Pulmonary and bony metastases following diagnosis (no adjuvant chemotherapy)	34		12	2500, 5000 mg/m^2	Static disease (12 mos)
Multifocal osteosarcoma	35	Femora, tibiae, humeri, skull (11 yrs following bilateral retinoblastoma at age 6 mos)	4	5000 mg/m^2	Static disease (3.5 mos)

A 9-year-old girl (patient 24) with primary osteosarcoma of the scapula with massive extension to the soft tissues of the shoulder area had no response following two treatments with Adriamycin-cyclophosphamide and one treatment with HDMTX; a parent refused further therapy for the patient. A 12-year-old girl (patient 25), who 11 years earlier had received radiation therapy to the orbit for retinoblastoma, had no response following three treatments with Adriamycin-cyclophosphamide and HDMTX at 2500 and 5000 mg/m^2. A 24-year-old woman (patient 26), who had received previous mantle radiation therapy for Hodgkin's disease and later developed osteosarcoma of the medial one third of the left clavicle, was treated with Adriamycin-cyclophosphamide-HDMTX for 1 year. The cumulative Adriamycin dose was limited to 300 mg/m^2; HDMTX was administered at a dose of 2500 mg/m^2 once monthly. This patient received ten monthly cycles of treatment. She developed a local recurrence in the mid-cervical region 40 months after diagnosis.

Of five patients (Nos. 27–31) with pulmonary metastases at diagnosis, response was obtained in one patient (No. 31) who at diagnosis had evidence of a single left lower lobe metastasis and right pneumothorax; this patient's response persisted for 5 months before the development of an additional metastasis in the left lower lobe area. The four additional patients (Nos. 27–30) failed to respond.

Adriamycin-cyclophosphamide-HDMTX was administered to a 6-year-old boy (patient 32) with Down's syndrome and a primary distal femoral osteosarcoma with nodal metastases in the thigh. This patient developed fatal MTX toxic effects following his second treatment (5000 mg/m^2) and had not demonstrated tumor response at the time of his death (13,15).

A 16-year-old girl (patient 33), who developed bilateral pulmonary and right cervical node metastases following right forequarter amputation for osteosarcoma detected during the third trimester of her first pregnancy, was treated with Adriamycin-cyclophosphamide-HDMTX which resulted in partial response of all measurable lesions for a period of 5 months (10,16). With evidence of progressive enlargement of the nodal metastasis, this lesion was resected and HDMTX administration was continued for an additional 6 months before there was evidence of uncontrolled growth of the pulmonary metastases and recurrent nodal metastasis.

Another patient (No. 34), who had amputation for osteosarcoma of the femur during pregnancy, had evidence of pulmonary, rib, and lumbar spine metastases 5 months after amputation (16). She had received no previous adjuvant chemotherapy and therefore received Adriamycin-cyclophosphamide-HDMTX for 1 year during which time her disease remained static. Treatment was discontinued after she developed truncal paralysis.

An 11-year-old girl (patient 35), who developed multifocal osteosarcoma 11 years following the diagnosis of bilateral retinoblastoma (17), had static disease for 3.5 months with the use of these agents, including HDMTX at 5000 mg/m^2.

Two patients (Nos. 36 and 37), without evidence of measurable indicators of disease activity, received adjuvant Adriamycin-cyclophosphamide-HDMTX (2500 mg/m^2 at monthly intervals) after resection of pulmonary metastases (table 4). One patient (No. 36), who had had a single central right pulmonary metastasis detected 15 months after amputation for femoral osteosarcoma, has had no evidence of recurrent disease for > 48 months after metastasectomy. One patient (No. 37), whose primary tumor of the distal tibia was associated with a left lower lobe pulmonary metastasis, had below-the-knee amputation and pulmonary metastasectomy immediately following diagnosis. This patient received five treatments

TABLE 4.—Adriamycin-cyclophosphamide-HDMTX for osteosarcoma patients without measurable indicators of disease activity

Patient No.	Treatment status	Clinical status or disease involvement	No. of combination treatments	HDMTX dose	Response after chemotherapy
36	Adjuvant therapy following removal of pulmonary metastasis	Amputation 15 mos prior to removal of single central metastasis	7	2500 mg/m²	No recurrence 4 yrs after metastasectomy
37	Adjuvant therapy following amputation and removal of pulmonary metastasis	Primary distal tibia	5	2500 mg/m²	No evidence of disease for 5 mos, then other pulmonary metastases

TABLE 5.—Summary of MTX pharmacokinetic data

Dose (mg/m²)	No. of patients/No. of observations	Half-life* (hrs)	C_0† (μM)	C_{24}‡ (μM)	Vd§ (liters/kg)
2500	4/4	2.7 ± 0.56	193 ± 23	0.41 ± 0.29	0.76 ± 0.19
5000	10/12	2.8 ± 0.46	420 ± 180	1.1 ± 1.9	0.88 ± 0.35
7500	9/23	2.7 ± 0.29	610 ± 320	1.9 ± 0.6	0.66 ± 0.31
10,000	2/2	2.9 ± 0.2	1220 ± 360	2.6 ± 1.6	0.47 ± 0.1
15,000	3/6	2.7	1400 ± 450	2.5 ± 0.88	0.54 ± 0.14

*Half-life for decline in serum concentrations from end of infusion until 24 hrs after infusion.
†C_0 = serum concentrations at end of infusion.
‡C_{24} = serum concentrations 24 hrs after end of infusion.
§Vd = apparent volume of distribution at end of infusion.

with adjuvant therapy and remained free of evidence of disease for 5 months before developing other pulmonary metastatic lesions.

METHOTREXATE PHARMACOKINETICS

Table 5 presents a summary of the pharmacokinetic data on 28 patients who received 2500–15,000 mg/m² of MTX during the treatments described in the previous sections. The half-life for decline in serum concentrations was similar for all doses of MTX, and serum concentrations at the end of MTX infusions and at 24 hours after infusion were directly related to the delivered dose of MTX, up to 10 g/m² (fig 1).

DISCUSSION

We have previously reported some of the results of our chemotherapy programs for adjuvant treatment of osteosarcoma patients (9,10) and for nonadjuvant therapy for patients whose treatment required consideration of their additional complicating features (10,11,13,16,17). This report details the treatment of this latter group of patients and cites details of the quality and duration of their responses.

Ten osteosarcoma patients with measurable primary or metastatic disease received treatment with HDMTX (1250–15,625 mg/m² at 1–2-week intervals). One of the ten patients had clearing of pulmonary metastases for 3 weeks, two developed partial response of pulmonary metastases which persisted for 7 months, and one with pleural metastases had a decrease in pleural effusion while receiving weekly HDMTX. No response was obtained with HDMTX at doses of 5000–7500 mg/m² in four patients who had received prior treatment with 2500 mg/m² of HDMTX as adjuvant chemotherapy. Of six patients rendered "apparently disease free" following resection of pulmonary metastases which developed during adjuvant Adriamycin-cyclophosphamide-HDMTX, administration of increased doses of HDMTX at 2–3-week intervals did not prevent the development of additional pulmonary metastatic nodules in five patients.

Thirteen patients with measurable primary or metastatic osteosarcoma received Adriamycin-cyclophosphamide-HDMTX (2500–5000 mg/m² in 4-week cycles). Two patients with incomplete resection of tumor of flat bones developed apparent complete responses for 7 and 40 months respectively; two patients with bulky residual disease after biopsy failed to respond. Nine patients with pulmonary, pulmonary-nodal, pulmonary-bony, primary-nodal, or multifocal primary osteosarcoma received sim-

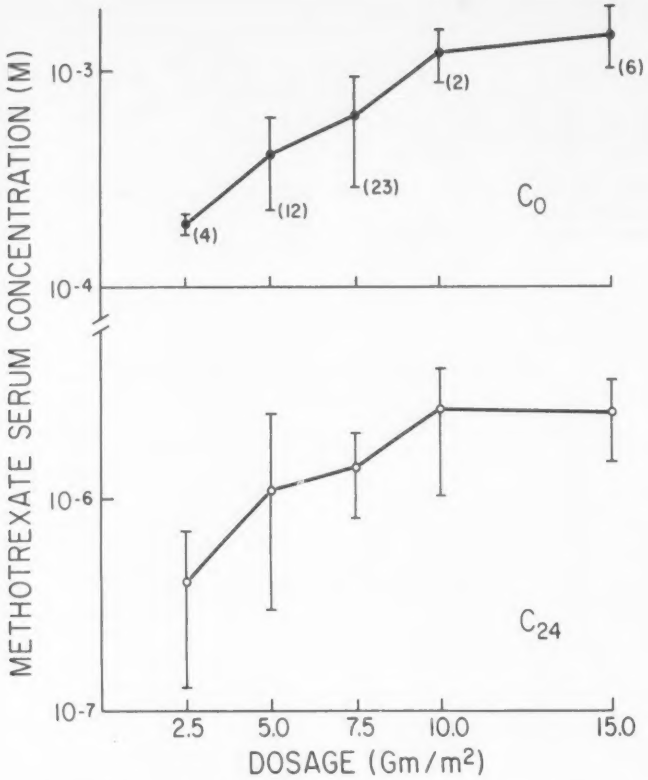


FIGURE 1.—Mean (± standard deviation) MTX serum concentrations observed at the end of a 6-hr infusion (●) and at 24 hrs after infusion (○) for doses ranging from 2.5 g/m² to 15 g/m². No. in parentheses = No. of MTX doses from which the data were derived.

ilar treatments; responses included one complete response for 5 months, one partial response for 5 months, and static disease in two patients for 3 and 13 months. Of two patients without evidence of residual tumor following resection of single pulmonary metastases, one remained free of disease for 5 months and the other continues to be free of tumor > 4 years from the start of adjuvant chemotherapy.

In 1972, Jaffe (1) reported response of pulmonary metastases in four of ten patients with metastatic osteosarcoma following treatment with HDMTX and leucovorin, according to the method of Djerassi et al (18). Two of the four patients had complete responses and two had partial responses.

The original doses of MTX used by Jaffe were 100 mg/kg (2500–3000 mg/m²) (1). When the indicator lesions failed to respond at this dose level, MTX doses were increased to 200 mg/kg or higher. Response of indicator lesions was reported with the higher doses (200–250 mg/kg, or 5000–6000 mg/m²) after failure of response with the relatively lower doses. It was also noted that many of the patients reported in the historical control group of osteosarcoma patients treated at the Boston Children's Hospital before 1972 had received treatment with lower "conventional" doses of MTX.

Jaffe et al (4) later observed that vincristine-HDMTX-leucovorin could be administered with safety on a weekly schedule for 4–8 weeks, within a setting of intensive pharmacologic and toxicologic monitoring. More recently it was reported that 15 of 21 patients developed complete responses with this weekly vincristine-HDMTX-leucovorin regimen, and 14 had no evident disease for 2+ to 18+ months; ten of these latter 14 patients remained free of disease from 9+ to 54+ months (19). The starting weekly dose of MTX recommended by Jaffe for treatment of overt metastatic osteosarcoma is 7500 mg/m², with in-

creases in doses as necessary until development of response or failure.

Rosen and associates reported the response of patients with metastatic osteosarcoma following Adriamycin-cyclophosphamide-vincristine-HDMTX-leucovorin treatments (5,6,20). Similar yet more intensive chemotherapy was combined with surgical resection of pulmonary metastases in their recently reported patient series (20), rendering more patients disease-free for longer periods of time than might be expected with single or repeated surgical resections of pulmonary metastases without chemotherapy. Rosen has recommended increasing the doses of MTX to the maximally tolerated doses, and to doses needed to obtain tumor response; these doses at times may be in excess of 15,000 mg/m^2.

The use of HDMTX alone or in combination with other agents known to be effective against measurable metastatic osteosarcoma has been accepted with little information as to its effectiveness as a single agent, and its use has been extended to patients with non-Hodgkin's lymphoma and acute lymphocytic leukemia (21). Studies of its effect on sarcomas, carcinomas of the lung, pancreas, and colon, and childhood tumors are in progress (22). In an attempt to generate beneficial information on this expensive and potentially toxic treatment method and to confirm the evidence for the effectiveness of HDMTX as a treatment method for osteosarcoma, our present studies involving newly diagnosed osteosarcoma patients with unresectable primary or metastatic lesions evaluate the effect of HDMTX in increasing doses, assessing the response of these measurable lesions prior to the addition of other chemotherapeutic agents such as Adriamycin and *cis*-dichlorodiammineplatinum(II).

While the dose and frequency of administration of HDMTX to our patients with osteosarcoma differed somewhat from the doses used in other centers, the doses were escalated when possible in an attempt to obtain tumor response. Few of our patients developed prolonged responses, whereas some patients who had been rendered disease-free following surgery have had responses of longer durations. The tumors of most patients who failed to respond following HDMTX also failed to respond following treatment with other apparently active agents.

In relating the efficacy of HDMTX to other chemotherapeutic agents used in the treatment of osteosarcoma, comparison of the results of Jaffe et al (19) with the results of Cortes et al (23) must be made. When vincristine-HDMTX was used by Jaffe, a 42% disease-free survival was projected by life-table analysis. Cortes' study of adjuvant Adriamycin has yielded a projected 39% 5-year survival rate; this result includes patients with "protocol violations." Early results of another Cancer and Leukemia Group B study indicated no difference in results of adjuvant Adriamycin in comparison to Adriamycin-HDMTX (24). Early results of the adjuvant use of Adriamycin with *cis*-dichlorodiammineplatinum(II) also appear encouraging (25).

Patients who developed pulmonary metastases while receiving adjuvant combination chemotherapy failed to develop responses after receiving increased doses of HDMTX at more frequent intervals than were used during adjuvant chemotherapy. As with other completely sensitive and partially sensitive tumors, osteosarcoma has been treated more successfully when there has been minimal tumor burden. This may account for the greater success of the adjuvant chemotherapy in comparison to the results of treatment of patients with unresectable primary or overt metastatic osteosarcoma.

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Antitumor Activity of Benzaldehyde [1]

Mutsuyuki Kochi,[2,*] Setsuo Takeuchi,[3] Takeo Mizutani,[3] Ko Mochizuki,[2] Yoshiaki Matsumoto,[2] and Yoshiharu Saito [2,4]

SUMMARY

Ninety patients with inoperable carcinoma in the terminal stages and 12 patients in serious condition with other tumor types were given benzaldehyde in the form of β-cyclodextrin benzaldehyde inclusion compound (CDBA) orally or rectally at a daily dose of 10 mg/kg divided in four doses. Toxic effects, including hematologic or biochemical disturbances, were not seen during long-term successive administration of CDBA. Fifty-seven of the patients treated were evaluable; 19 patients responded completely and ten patients responded partially (> 50% regression). For all responding patients longer response durations were associated with longer CDBA treatment periods. Treatment of squamous cell carcinoma induced the cancer cells to change into a conglomeration of pearls (the well-known product of differentiation) which consisted of keratinized normal squamous cells.

[Cancer Treat Rep 64:21–23, 1980]

We have previously shown that the volatile fraction absorbed by active charcoal from the fig has effective activity against Ehrlich carcinoma of mice. Therefore, between 1965 and 1975, 83 cancer patients were treated iv with the volatile fraction of the fig. This fraction proved to be effective in 12 patients, four of whom responded completely, while no appreciable effect was noted in the other 71 patients. Having achieved an appreciable effect with the volatile fraction of the fig, we studied the carcinostatic component of the fig and identified the effective agent as benzaldehyde (1). Benzaldehyde has shown some antitumor activity against Ehrlich carcinoma, adenocarcinoma 755, and spontaneous hepatoma in mice; however, it did not have activity against several other implanted tumors of mice (1).

METHODS

Since benzaldehyde is only slightly soluble in water, it is not suitable for iv, im, or sc injection. The β-cyclodextrin benzaldehyde inclusion compound (CDBA) ($C_{49}H_{76}O_{36}$) is a preparation suitable for both oral and rectal administration. CDBA was used mainly in the form of a tablet or suppository. The patients were treated with 10 mg/kg of CDBA in four divided doses a day. The amount of benzaldehyde contained in CDBA is approximately 8.3%, therefore 6 g of CDBA was required in order to provide 500 mg of benzaldehyde.

RESULTS

All patients had histologic confirmation of their diagnosis. No known curative treatment existed for these patients, and there was no palliative treatment which we thought would be of benefit to them. All but four patients had received previous chemotherapy, and 15 had received prior radiotherapy. The patients were observed daily for toxicity and side effects. Hematologic and biochemical examinations were performed once a week. Baseline studies included physical examination, measurement of body weight and height, cbc, blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, SGOT, uric acid, serum protein, albumin, urinalysis, electrocardiogram,

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TABLE 1.—Spectrum of diseases treated with benzaldehyde and response to therapy

	No. of patients	No. of responses				
		Complete	Partial	Improvement	Stable disease	Progression
Carcinoma						
Tongue	4	4				
Parasinus	1		1			
Parotid	1	1				
Lung	9	3	3		1	2
Breast	2	1		1		
Esophagus	2		1		1	
Stomach	10	2		8		
Liver	6		2	3	1	
Pancreas	4	1		2	1	
Colon	1	1				
Rectum	3	1		2		
Testis (seminoma)	1				1	
Kidney (Grawitz's tumor)	2			2		
Brain	3	1			2	
Gall bladder	1	1				
Transitional cell	1		1			
Acute myelocytic leukemia	2	2				
Malignant lymphoma	2		1	1		
Multiple myeloma	1	1				
Leiomyosarcoma	1		1			
Total	57	19	10	19	7	2

and measurement of tumor size. Bone marrow aspirations were done in leukemic patients.

Fifty-seven evaluable patients, 32 men and 15 women, with far-advanced malignant neoplasms were included in the study. They ranged in age from 4 to 83 years with a mean age of 53 years. The spectrum of diseases treated with CDBA is detailed in table 1. The clinical study with CDBA started 2 years and 5 months ago. All patients were observed for periods ranging from 2 weeks to > 2 years. Three of four patients with squamous cell carcinoma of the tongue had received prior radiotherapy and chemotherapy. These patients were all in serious condition at the start of the treatment. After 1.5–6 months of treatment with CDBA, all patients with tongue cancer obtained complete remission. It is very interesting that the cancer cells of these four patients were extremely differentiated histologically and changed into a conglomeration of pearls which consisted of keratinized normal squamous cells. Another patient with squamous cell carcinoma of parasinus and lung metastases obtained a partial response after 3 months of treatment with CDBA. The lung tumors almost disappeared and the gigantic tumor of the temporal area also markedly improved. In this patient, the cancer cells were also changed into a conglomeration of pearls which consisted of keratinized normal squamous cells. The responses of these five patients continued during the successive treatment with CDBA. There were no recognizable toxic effects from the treatment. An 83-year-old woman with adenocarcinoma of the rectum underwent an operation for an artificial anus because of the complete intestinal obstruction resulting from the rectal tumor. She had not received prior radiotherapy or chemotherapy. She responded completely and the response lasted 2 years and 1 month during the successive treatment with CDBA. As a result of this response stools now pass through the natural anus and the patient is enjoying normal life. Moreover, no recognizable toxic effects were observed. Here, we found that the responsive adenocarcinoma cells were differentiated and changed into ghost cells simultaneously. A 4-year-old boy with acute myelocytic leukemia previously received a 10-month treatment with Adriamycin, cytosine arabinoside, vincristine, and prednisolone, with methotrexate as maintenance therapy, but no complete remission occurred. Ten days after the initiation of the treatment with CDBA, he obtained complete remission and his platelet count, leukocyte count, and hemoglobin level returned to normal. The complete remission has lasted > 4 months so far, and there were no recognizable toxic effects during the treatment.

Despite persistent oral administration with CDBA (approximately 500 mg/day of benzaldehyde) for > 1

year, it has proved nontoxic and does not adversely affect hepatic or renal functions, nor does it cause side effects such as leukopenia, thrombocytopenia, oligocythemia, anorexia, vomiting, and depilation, many of which are associated with cytotoxic anticancer agents. The responses continued during the treatment with CDBA, but the optimal dose of benzaldehyde has not yet been established. However, various types of tumor cells indicated varying degrees of sensitivity to benzaldehyde. The dose of 30 mg/day proved to be remarkably effective against leiomyosarcoma. However, the effective dose for squamous cell carcinoma or adenocarcinoma was found to be > 300 mg/day. We suggest that CDBA should be further evaluated with regard to both mechanism of action and clinical efficacy.

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Initial Clinical Study With Vindesine: Tolerance to Weekly Iv Bolus and 24-Hour Infusion [1,2]

Takao Ohnuma,* Ezra M. Greenspan, and James F. Holland [3,4]

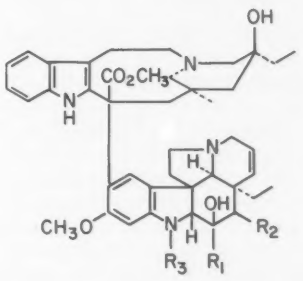
SUMMARY

Forty-six patients with inoperable cancer and leukemia in relapse were given vindesine (VDS) either by iv bolus weekly at doses ranging from 2.0 to 5.5 mg/m² or by 24-hour continuous infusion weekly at doses ranging from 1.0 to 7.0 mg/m² of estimated body surface area. VDS was well-tolerated by patients with normal liver function who had previously been minimally treated with myelosuppressive agents at a dose of ≤ 4 mg/m² either by iv bolus or by 24-hour infusion weekly. The dose-limiting toxic effects of VDS were leukopenia and neurotoxicity. Leukopenia was cumulative but easily reversible by interruption of weekly dose. Neurotoxicity was insidious and hardly reversible. Patients with liver dysfunction appeared to develop more neurotoxicity. Other toxic effects included a decrease in hemoglobin level, transient hepatic dysfunction, cellulitis or phlebitis at the iv site, stomatitis, nausea, and vomiting. Degrees and parameters of toxic effects observed after iv bolus and 24-hour infusion of the same doses were indistinguishable except for an increased incidence of local cellulitis in the infusion group.

[Cancer Treat Rep 64:25–30, 1980]

Vindesine (VDS) is a new synthetic product derived from vinblastine (fig 1), a vinca alkaloid. Animal studies revealed that the antineoplastic spectrum of VDS against a murine transplantable tumor system resembled that of vincristine (VCR), but the former compound appeared to lack the dose-limiting neurotoxicity of the latter compound (1).[5] Comparative toxicity studies in mice and rats

STRUCTURAL FORMULA



	VINDESINE	VINBLASTINE	VINCRISTINE
R_1	$CONH_2$	$COOCH_3$	$COOCH_3$
R_2	OH	$OCOCH_3$	$OCOCH_3$
R_3	CH_3	CH_3	CHO

FIGURE 1

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[4]We thank Drs. H. Bruckner, P. Chahinian, S. Cohen, J. Cuttner, M. Goldsmith, L. Ratner, and S. Waxman for including their patients in this study, and Sandra Brooks, RN, for providing skillful nursing care.

[5]Information for clinical investigators: Compound 99024, desacetyl vinblastine amide sulfate, an investigational new drug. Prepared by the Eli Lilly and Co, Indianapolis, IN, Apr 14, 1975.

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TABLE 1.—Disease categories

Disease categories	Iv bolus	24-hr infusion	Total
Diagnosis			
Breast carcinoma	8	4	12
Lung carcinoma	6	1	7
Melanoma	2	3	5
Sarcoma	1	3	4
Ovarian carcinoma	2	1	3
Kidney carcinoma	0	3	3
Acute myelogenous leukemia	0	3	3
Colon carcinoma	2	0	2
Miscellaneous	5*	2†	7
Total	26	20	46

*1 each with medullary carcinoma of thyroid, adenocarcinoma of parotid gland, sweat gland, and rectum, and Schmincke tumor.

†1 each with blastic crisis of chronic myelogenous leukemia and leukemic phase of lymphosarcoma.

showed that VDS was more toxic than vinblastine but less toxic than VCR. Toxic manifestations included anorexia, cytopenia, and atrophic intestinal mucosa (2).

Phase I clinical trials of VDS were carried out with weekly iv bolus or daily injection schedules (3–6). VDS was found to be both neurotoxic and myelosuppressive in man.

In our phase I trial we evaluated the clinical effects of VDS given by iv bolus and by continuous 24-hour infusion. Our interest in VDS infusion stems from our prior experience with VCR infusion where we observed rather striking therapeutic effects in a small number of patients (7). In our recent study of neocarzinostatin we were able to demonstrate differences in parameters of dose-limiting toxicity between the 2- and 24-hour infusion schedules (8). Portions of this present work appeared in a preliminary form elsewhere (9).

MATERIALS AND METHODS

VDS was supplied by Dr. Richard Dyke of the Lilly Laboratories for Clinical Research (Indianapolis, IN) as a lyophilized powder at a dose of 10 mg in a 10-ml ampule. The ampules were stored under refrigeration. Just prior to use, the drug was diluted in a bacteriostatic sodium chloride injection solution and was administered as either a slow iv bolus through the side arm of a running infusion or a 24-hour infusion. In each schedule, the treatment was repeated weekly or as often as tolerated.

Forty-six patients (26 males and 20 females) with advanced malignant neoplasms were included in the study. Their ages ranged from 14 to 74 years (median, 52.5 years; mean, 51.8 years). All patients had cancer or leukemia which was microscopically confirmed (*see* table 1 for disease categories) and considered unresponsive to conventional therapy. All patients had been treated previously with chemotherapy and 15 had also received radiotherapy. Seventeen patients had had prior VCR treatment. Patient performance status was classified according to the Cancer and Leukemia Group B (CALGB) criteria as follows: 0 = normal performance (0 patients); 1+ = ambulatory (ten); 2+ = < 50% of waking time in bed (nine); 3+ = > 50% of waking time in bed (14); and 4+ = 100% bedridden (13). Nine patients with pre-existing hepatic dysfunction (bilirubin ≥ 2.0 mg/dl without clinical and laboratory evidence of hemolysis and SGOT ≥ 100 milliunits/ml) due to neoplastic involvement were entered in the study in order to assess the effects of the dysfunction on toxicity. Informed consent was obtained from the patients or their legal guardians prior to initiation of the study.

Study Procedure

Baseline studies included physical examination, body weight and height, cbcs and complete automated chemical profiles (SMA-6 and -12), urinalysis, chest roentgenogram, and measurement of tumor lesions, if present. Bone marrow examinations were carried out in all patients with leukemia and in some patients with solid tumors.

The initial dose of 2.0 mg/m^2/iv bolus weekly was adopted from a similar study carried out at the Sloan-Kettering Memorial Hospital (New York, NY) indicating that the dose appeared to be the highest nontoxic or minimally toxic dose in man (I. Krakoff, personal communication). The dose was escalated in increments of 50% and 33% . . . of the preceding step. This cautious escalation was based on the encountering of ill-defined neurotoxicity in an early phase of the study of this drug. After determining the parameters of toxicity in the bolus schedule, we studied a continuous iv infusion over 24 hours in patients who were hospitalized and in patients with leukemia. The dose was started at 1.0 mg/m^2 and was escalated by a modified Fibonacci search scheme (10).

TABLE 2.—Dose escalation and hematologic toxicity (nonhematologic neoplasms only)

Dose (mg/m²/wk)	No. of patients	No. of doses given	No. of patients with toxic effect/total evaluable: Decrease of hemoglobin (> 1.5 g/dl)	No. of patients with toxic effect/total evaluable: Leukopenia (wbc count < 4000/μl)	Median (range)—: Lowest wbc count recorded* (cells × 10³/μl)	Median (range)—: Day
			Bolus			
2.0	3	2,2,8	3/3	2/3	2.8,2.9	6,13
3.0	3	3,7,13	2/3	3/3	3.6(1.3–3.6)	25(15–36)
4.0	13	1,1,1,1,2,2,2,2,2,3,5,6,6	5/12	8/12	2.5(1.75–3.9)	13(5–72)
5.0	7	1,1,2,4,6,10,14	2/5	4/7	0.8(0.225–2.9)	18(6–61)
Total	26		12/23	17/25		
			24-hr infusion			
1.0	2	1,2	0/1	0/1		
2.0	2	3,4	0/2	0/2		
3.3	2	1,2	0/1	—		
4.0	7	1,1,1,1,1,2,2	2/4	6/7	2.45(0.875–3.9)	9(4–16)
5.0	6	1,1,1,1,2,2	2/2	3/4	2.3(0.4–3.7)	8(7–9)
7.0	1†	1	—	—		
Total	20		4/10	9/14		

*Wbc count of < 4000/μl only.
†Not evaluable (*see* text).

In the latter part of the study, patients were entered at the 4.0-mg/m² dose level in attempts to compare the clinical toxicity of the two schedules.

Responses in solid tumors in patients with measurable diseases were evaluated according to our current criteria (8). The criteria of response in patients with leukemia were based on those adopted by CALGB.[6]

RESULTS

Of 46 patients treated with VDS, 26 received the drug by iv bolus weekly and 20 by continuous 24-hour infusion weekly. Two patients who received VDS as an iv bolus for the first course of treatment received the second course as a 24-hour infusion for the purpose of pharmacologic study. One patient received VDS as a 24-hour infusion for the first course and as an iv bolus for the subsequent courses. The dose was not escalated in the same patient. Four patients died within 2 weeks of treatment. One patient with blastic crisis of chronic myelogenous leukemia (CML) and another with acute myelogenous leukemia died on Day 5, clearly from disease-related complications, after receiving 5.0 mg/m² of VDS as a 24-hour infusion. A third patient, with kidney carcinoma with mediastinal involvement, developed pulmonary edema and died on Day 3 after receiving 7.0 mg/m² of the drug as a 24-hour infusion and, on Day 2, 166 rads of radiation to the mediastinal tumor which had been causing esophageal stenosis and dysphagia; any role of VDS in this premature death could not be assessed retrospectively. Toxicity was not evaluated in these three patients. The fourth patient, with spindle cell sarcoma of the neck, died on Day 12 from progressive disease. The remaining patients were observed for 2–17 weeks.

Toxicity

The major toxic effects of VDS were anemia, leukopenia, and neurologic complications (tables 2 and 3). A decrease of > 1.5 g/dl in the hemoglobin level occurred in about one half of the patients in whom no hemoglobin fall had been recognized during pretreatment observation periods. Leukopenia was dose-limiting; administration of a 5.5-mg/m² iv bolus resulted in leukopenia of < 1000 cells/μl in two of seven evaluable patients and in sepsis in one patient who was then hospitalized. Leukopenia was cumulative but rapidly reversible with a weekly bolus schedule. Thus, of 12 patients who received more than three weekly courses, the course was interrupted (with wbcs of < 2000/μl) or reduced by one half (with wbcs of 2000–4000/μl) after the second course in two patients, after the third course in five, and after the fourth course in two. In three patients the interruption of the course due to leukopenia resulted in a "2 weeks on, 1 week off" pattern.

Neurologic toxic effects were essentially similar to those which occur after VCR: primarily peripheral paresthesia and decreased deep-tendon reflexes (table 3). A

[6]Criteria for Evaluating Acute Leukemia of the Cancer and Leukemia Group B are available from the CALGB Operations Office, 2 Overhill Rd, Suite 208, Scarsdale, NY 10583.

TABLE 3.—Dose escalation and neurologic toxicity

Dose (mg/m^2)	No. of patients			No. of patients with toxic effect/No. evaluable									
	Total treated	With previous VCR	With pre-existing hepatic dysfunction	Muscle weakness	Myalgia	Paresthesia	Decreased deep-tendon reflexes	Parotid pain	Constipation	Hoarseness	Headache	Confusion, delirium, hallucination	Total
Bolus													
2.0	3	2	2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2
3.0	3	1	2	3/3	0/3	2/3	3/3	0/3	0/3	0/3	0/3	1/3	3/3
4.0	13	5	2	7/10	1/10	5/8	6/8	0/10	0/10	0/10	1/10	2/10	7/10
5.5	7	2	1	5/5	4/6	2/4	4/4	3/6	1/6	1/6	0/6	1/6*	6/6
Total	26	10	7	16/20	6/21	9/17	13/17	3/21	1/21	1/21	1/21	4/21	17/21
24-hr infusion													
1.0	2	0	0	0/2	0/2	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
2.0	2	0	0	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
3.3	2	1	0	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
4.0	7	4	1	4/6	0/6	3/6	5/6	1/6	1/6	0/6	0/6	0/6	5/6
5.0	6	2	1	3/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4	1/4	4/4
7.0	1†	0	0										
Total	20	7	2	7/15	0/15	3/15	7/15	1/15	2/15	0/15	0/15	0/15	10/15

*The patient with hallucination also received Thorazine.
†Not evaluable (*see* text).

TABLE 4.—Reasons for discontinuation of treatment

	No. of patients	
	Bolus	24-hr infusion
Progressive disease or no response	9	10
Discharged to other physician or institution	5	2
Neurologic toxicity	5	0
Leukopenia	2	4
Death	2	3
Lost to followup	2	0
Hepatic toxicity	1	0
Cellulitis	0	1
Total	26	20

majority of patients also complained of generalized muscle weakness. With prolonged courses of chemotherapy, nearly all patients developed neurotoxic effects. At the highest dose studied, early myalgia and muscle weakness predominated. Parotid or jaw pain, transient constipation, hoarseness, confusion, and hallucination were also seen in a small number of patients. Two patients refused treatment after the first injection of VDS, one because of troublesome neurotoxicity and the other because of cellulitis. The reasons for discontinuing treatment are shown in table 4.

At low doses of 2.0~3.0 mg/m²/week by iv bolus, all of the three evaluable patients with hepatic dysfunction developed profound muscle weakness and/or myalgia within three courses of treatment and two of the three developed paresthesia. In contrast, two patients with normal hepatic function had no muscle weakness or myalgia; one patient developed general weakness only after the 11th course of treatment and paresthesia after the 14th course. These results strongly implicated overt hepatic dysfunction as a risk factor.

VDS infusion produced essentially similar parameters of toxicity. Thrombocytopenia was noted in one of the patients in the infusion group and was of no major consequence. Since this study was not designed to compare the toxicity of iv bolus versus 24-hour infusion, and because the two groups of patients were dissimilar (performance status 1 and 2, 50% versus 30%; pre-existing hepatic dysfunction, 27% versus 10%; patients with more than three courses, 12 versus two), the data were not ideal for direct comparison of the toxic effects of the two schedules. Nevertheless, both schedules appear to have produced similar degrees of leukopenia (the median nadir of leukopenia after the first course of 4 mg/m² of VDS was 4600 cells/μl [range, 1175~6200] versus 3600 cells/μl [range, 1000~5400], $0.5 > P > 0.1$). Neurologic toxicity was insidious and was primarily a function of cumulative dose received, which made quantitative comparison of the two schedules difficult. The lower incidence of neurologic complications seen in the infusion group (table 3) is most likely related to the lower cumulative dose given to, and lesser number of patients with functional hepatic abnormalities in, this group. These considerations in mind, it was an impression of clinical investigators that the degrees of hematologic and neurologic toxicity observed after iv bolus and 24-hour infusion of the same dose of VDS were indistinguishable. Four patients receiving the infusion schedule developed cellulitis at the infusion site in contrast to only one patient experiencing cellulitis after receiving the bolus schedule.

Seventeen patients with prior exposure to VCR received VDS. Since some of these patients already had decreased deep-tendon reflexes and the number of evaluable patients in each dose level was small, no conclusion could be drawn from the study with regard to whether prior exposure to VCR had any effects on VDS neurotoxicity.

Other toxic effects included transient hepatic dysfunction which was manifested as transient increase in bilirubin and transaminases in five of 23 patients after iv bolus and in two of 15 after infusion, nausea and vomiting in two patients after bolus, and stomatitis in one patient after bolus.

Hematologic Effects in Patients With Leukemia

Hematologic effects of VDS were evaluated for six courses of infusion given to three patients with acute myelogenous leukemia in relapse and to one with blastic crisis of CML. A decrease in circulating peripheral blast cells was noted on three occasions and the bone marrow became hypocellular at a dose of 5 mg/m²/24 hours. No patient had bone marrow improvement, however.

Therapeutic Effects

VDS treatment did not induce any partial or complete responses among our series of patients. One patient with Schmincke's disease involving bilateral neck nodes had an approximately 25% reduction in tumor size lasting about 1 month after receiving 4.0 mg/m² of VDS by iv bolus weekly. One patient with adenocarcinoma of the rectum and two patients with metastatic breast carcinoma had stable disease lasting 3~4½ months. Treatment was discontinued in the latter three patients because of progressive neurotoxicity.

DISCUSSION

VDS, shown to have an antitumor spectrum similar to VCR without VCR's dose-limiting neurotoxicity in the preclinical study, was recognized to produce both dose-limiting leukopenia and neurotoxic effects in man. Other

toxic effects included anemia, transient hepatic dysfunction, local cellulitis and/or phlebitis, nausea, vomiting, and stomatitis. Our observation that the dose-limiting toxic effects of VDS were leukopenia and neurotoxic effects was in accord with earlier preliminary observations by other investigators. Our initial dose of 1.0 mg/m^2 used in the 24-hour infusion schedule was based on our own data in iv bolus schedule and on the preliminary clinical experience at the Lilly Laboratories for Clinical Research where a dose of 0.5 mg/m^2 weekly for 17 weeks in one patient with multiple myeloma produced no objective or subjective effects. A dose of 2.5 mg/m^2 weekly for three doses in a patient with carcinoma of the lung resulted in leukopenia (R. Dyke, personal communication). In toxicologic studies in dogs at Lilly, the toxic dose low was 0.02 mg/kg on a biweekly schedule (*see* footnote 5) which could be extrapolated to about 0.5 mg/m^2/week for a one-third toxic dose low in man.

Administration of 5.5 mg/m^2 of VDS iv bolus resulted in leukopenia of < 1000 cells/μl in two of seven patients after the first course with one leukopenic episode complicated by sepsis. In three of the remaining five patients, the schedule had to be modified to space the interval longer than weekly. From these findings, we concluded that we had reached the maximally tolerable dose. VDS at a dose of 4.0 mg/m^2 iv bolus appeared to be well-tolerated in the weekly schedule; two patients received six weekly courses and one had five weekly courses without dose-limiting toxicity and the treatment was discontinued because of progressive disease. Although the 24-hour infusion was tested only up to two courses, patients appear to have tolerated the treatment well with identical parameters and, probably, similar degrees of toxicity. The 24-hour infusion had to be given to patients who were hospitalized, and it was associated with an increased incidence of cellulitis at the iv site. Thus, from a clinical toxicologic viewpoint, the iv bolus route seems preferable. In a phase II trial Mathé et al (11) reported that a continuous infusion of VDS of 2 mg/m^2/24 hours for 2 days resulted in six complete remissions occurring among 15 patients with acute lymphocytic leukemia and three complete remissions occurring among six patients with blastic crisis of CML. Since we did not observe sufficient therapeutic activity to determine the therapeutic advantage of one schedule over another, no conclusion could be drawn from this study as to whether one schedule was therapeutically more active than the other. It may be of interest to carry out disease-oriented phase II trials at a dose of 4.0 mg/m^2 by iv bolus or 24-hour infusion weekly in patients with normal hepatic and marrow functions until development of dose-limiting neurologic toxicity.

In the early phase of the study we encountered profound muscle weakness in patients with hepatic impairment. It is possible that since VDS, like vinblastine, is excreted primarily through bile (12,13), removal of VDS from circulation will be delayed in patients with hepatic impairment with subsequent increase in neurologic toxicity.

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Intra-Arterial Infusion of Anticancer Drugs: Theoretic Aspects of Drug Delivery and Review of Responses [1,2]

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SUMMARY

A linear, physiologically based pharmacokinetic model has been used to study drug delivery characteristics of anticancer drugs after both iv and intra-arterial infusion. It confirms the general belief that intra-arterial infusion produces an increase in local tissue levels and a reduction in systemic drug availability. The increase in local drug concentration depends largely on the blood flow rate of the infusing artery and the rate of drug elimination by the rest of the body. A low arterial blood flow rate and a high drug elimination rate will ensure a high local drug level. On the other hand, the reduction of systemic drug delivery depends on the ability of the infused region to eliminate the drug. Lower systemic availability will be obtained if a substantial amount of the drug is removed during the first pass through the infused region. A review of the clinical response rates of five cytotoxic drugs (Adriamycin, bleomycin, 5-fluorouracil, melphalan, and methotrexate) infused intra-arterially as single agents indicates that, despite a general belief in the advantages of intra-arterial infusion, only 5-fluorouracil shows a significant increase in response rate in the treatment of cancer when compared to systemic routes of administration. There appears to be no justification for the higher costs and substantial risks of intra-arterial infusion for the other drugs due to the lack of controlled studies and the lack of a substantial increase in the response rates. It is suggested that a future controlled study be required to validate the claims of superiority of intra-arterial infusion therapy.

[Cancer Treat Rep 64:31–40, 1980]

Intra-arterial infusion has been used frequently for the treatment of localized malignant diseases. The general belief is that this technique increases the drug delivery to the infused area and simultaneously reduces the amount of drug going into the system's circulation. As a result, the tumor kill will be enhanced and the toxicity to the rest of the body will be minimized. The theoretic basis for the principles governing the advantages of intra-arterial infusions has been reported by Eckman et al (1). However, the paper seems to go unnoticed in practical application. Instead of basing their justification on the pharmacokinetics of the drug to be used and the organ to be infused, clinicians tend to justify the use of intra-arterial infusion based on the successful results of previous experiments in which different drugs and different organs were involved.

Part of the reason for the difficulty in applying the theoretic principles is probably the complicated mathematics involved and also the concept of the probability that a molecule of drug will be lost during circulation. Previous authors (1) defined

$$R_d = \frac{[\int_0^\infty C_i(t)dt]_{\text{arterial}}}{[\int_0^\infty C_i(t)dt]_{\text{venous}}} \quad [1]$$

as the delivery advantage to a particular site resulting from arterial infusion, where C_i is the drug concentration

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at time t. They showed that this advantage is independent of the rate of drug infusion and gave

$$R_d = \frac{1 + [(1-\alpha)/\alpha]\delta_s}{1 - \delta_L} \quad [2]$$

where α is the fraction of the cardiac output that flows through the infusing artery and δ_s and δ_L are the probabilities that a drug molecule will be lost during a single systemic circulation and during a passage through the lungs. They also gave the advantage of decreased systemic drug delivery through arterial infusion as

$$R_d = 1-\delta_i \quad [3]$$

where δ_i is the probability that a drug molecule will be lost during a passage through the infused region i.

In this paper, equations [2] and [3] will be derived from a physiologically based pharmacokinetic model. The probabilities δ_s, δ_L, and δ_i will then be related to the blood perfusion rates and the organ elimination rates. The present results based on these known physiologic and pharmacologic parameters will therefore be more suitable for practical application.

THEORETIC CONSIDERATION OF ADVANTAGES OF INTRA-ARTERIAL INFUSION

As shown in the Appendix, the physiologically based pharmacokinetic model (fig 1) can be used to study the advantages of intra-arterial infusion compared to iv infusion. In brief, the advantage of drug delivery to the tumor (or target region) through intra-arterial infusion can be expressed as

$$R_d = 1 + K_{app}/Q_T \quad [4]$$

where K_{app} is the total apparent drug clearance from the body, except for the tumor (target) region. Therefore, R_d is independent of the elimination capacity of the infusion region but depends on the rate of elimination from the rest of the body and the blood perfusion rate into the target region. The faster the drug is eliminated by the rest of the body and the smaller the blood perfusion rate to the tumor, the greater the advantage of drug delivery through arterial infusion will be. If the drug is eliminated by the infused organ only, then $R_d = 1$ and no advantage in drug delivery will be gained.

On the other hand, intra-arterial infusion will reduce drug delivery and availability to the rest of the body by

$$R_d = 1 - \frac{K_T}{Q_T+K_T} = 1 - E_T \quad [5]$$

where E_T is the extraction ratio of the drug by the target region. Thus, with a greater ability of the target organ to eliminate the drug, there is more reduction of drug delivery to the systemic circulation and a concomitant decrease in toxicity. If a drug is not eliminated by the infused area, there will be no reduction in systemic drug availability.

If the response or cancer cell kill and the toxicity to normal cells are both linearly proportional to the "exposure" or Concentration × time (C × t) of the drug, the advantages of increasing tumor response by using intra-arterial infusion will follow equation [4] and the reduction of systemic toxicity will be given by equation [5]. If only C × t is of importance, the rate of infusion and the duration of infusion will have no effect on the response and toxicity as long as the total dose remains constant. However, it is true that some drugs may need a minimum drug concentration to achieve cell kill, and that the cell

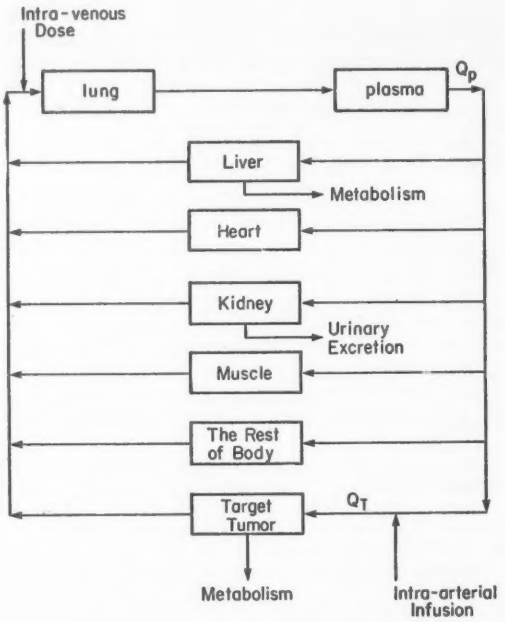

FIGURE 1.—Pharmacokinetic model for intra-arterial and iv infusion.

kill is proportional to its peak level in vivo. In this nonlinear case, the present theory does not apply. Nevertheless, as the rate of infusion increases and the duration of infusion decreases for constant dosage, the peak drug concentration will increase. Therefore, the intra-arterial bolus injection will offer the best response if the toxicity is still tolerable.

INTRA-ARTERIAL INFUSION OF ANTICANCER DRUGS

Adriamycin

The principal route of Adriamycin elimination in cancer patients is through biliary excretion and liver metabolism. Less than 5% of the administered dose is excreted unchanged in the urine (2). Patients with significantly impaired liver function had marked elevation and prolongation of plasma Adriamycin levels associated with severe toxicity unless the dose was reduced. It has also been shown (3) that hepatic portal vein infusion resulted in a 60% loss of Adriamycin on the first pass through the rabbit liver. The hepatic extraction ratio for Adriamycin in man is probably close to 0.6.

Therefore, it can be shown from the previous results that if Adriamycin is infused intra-arterially into a region (excluding the liver) with a blood flow rate of 100 ml/minute, the R_d advantage of increasing regional drug delivery will be 9.7 assuming Q_{liver} = 1450 ml/minute for a 70-kg patient. However, there will be no reduction in the systemic drug availability since this region does not eliminate the drug. On the other hand, if the organ receiving the infusion is the liver, there will be no increased drug delivery to the liver because all the drug will eventually go through that organ and be eliminated. However, the liver does clear 60% of the drug before it gets into the systemic circulation so there will be a reduction of 60% in the systemic Adriamycin availability. If the cancer cell kill and the toxicity are both linearly proportional to the value of Adriamycin $C \times t$ (integral of the concentration versus time curve) only, then compared with iv infusion the following responses are expected after arterial Adriamycin:

(1) If the region to receive the infusion is the liver, no improvement in tumor kill is expected but there should be a reduction in systemic toxicity.
(2) If an organ other than the liver receives the infusion, there should be an increase in the regional drug level and, hence, the tumor regression, but no reduction in systemic toxicity.

Adriamycin concentrations after both iv and intra-arterial administration have been compared recently in both dogs and man (4). Drug levels in regional tissues of dogs were not significantly different after intra-arterial infusion as compared to those after iv infusion. However, the drug levels in the extremity receiving intra-arterial infusions were generally somewhat higher than those of the contralateral extremity in the same group. Furthermore, the drug levels in the tissues of the contralateral extremity of the intra-arterial infusion group were the same as those of the levels in the iv infusion group. This confirms that a significant amount of Adriamycin went into the general circulation after regional intra-arterial infusion. In patients who received Adriamycin by the iv route, the drug levels in the peripheral blood were also comparable to those in the peripheral blood of patients receiving Adriamycin by intra-arterial infusion. The reason for the small differences in the drug levels in dog tissues after iv and intra-arterial infusion may be that the femoral artery which received the infusion has a rather high blood flow rate of 55 ml/minute. According to equation [4], a higher blood flow rate through the infused region reduces the advantage of increasing drug delivery. With a liver blood flow rate of 60 ml/minute and a hepatic Adriamycin extraction ratio of 0.6, R_d = 2.1, which is probably too low to expect any significant difference in the experiments.

Clinical application of intra-arterial infusion with Adriamycin in the treatment of malignant diseases has been popular (5–11) but the results are not conclusive. Compared with Adriamycin given iv, there were reports of similar antitumor effects (5,10) and similar (6,8–10) or higher (5) toxicity with the intra-arterial infusions. In most of the studies, the number of patients was too small and the different types of malignant diseases were not separated. The only study that actually compared intra-arterial infusion and iv bolus injection of Adriamycin reported no significant difference in either antitumor effect or toxicity for both groups of patients (10). However, there were only four evaluable patients in each group. Previous results (5–10) are separated into two categories: infusion into the hepatic artery and infusion into the rest of the body (table 1). Nevertheless, no significant difference in either antitumor effect (> 50% regression) or toxicity between these two types of regional infusions was reported. The only difference appears to be the total number of responses (including < 50% regression): 43% for hepatic infusion versus 62% for the rest of the body. This could indicate that liver cancers are less sensitive to Adriamycin.

There are several possible reasons for the discrepancy between the theoretic predictions and the clinical results:

(1) The large variations in response rates between different types of cancers may be why there is no superior antitumor effect for intra-arterial infusion of Adriamycin.
(2) Liver tumors may reduce the ability of the liver to

metabolize Adriamycin and, hence, hepatic arterial infusions of Adriamycin may yield no reduction in systemic toxicity.

(3) Hepatic clearance of Adriamycin is nonlinear and becomes saturated during hepatic arterial infusion.

(4) Adriamycin metabolites are responsible for the systemic toxicity.

Bleomycin

Bleomycin is eliminated from the human body primarily through urinary excretion. About 45%–70% of the administered dose is excreted unchanged in the urine 24 hours after iv bolus injection (12,13). The total body plasma clearance of this drug was estimated to be 87.5 ± 16.6 ml/minute (12). Therefore, arterial infusion of bleomycin should increase regional drug delivery slightly (R_d = 2.46, assuming Q_T = 100 ml/minute and the hematocrit value = 0.4) with no change in the systemic availability of the drug. One might expect that there will be only slight improvement in the antitumor effect and no change in toxicity as compared with other systemic routes of administration.

Clinical results show that the theoretic advantages of intra-arterial infusion of bleomycin were not of clinical importance (14–17). Huntington et al (14) treated 21 patients with inoperable squamous cell carcinomas of various sites using regional intra-arterial infusion of bleomycin. In five of 21 patients this method caused short-term but significant tumor regression, while the toxicity was minor and less than with other methods of bleomycin administration. Furthermore, the overall responses were also similar to those achieved by other systemic routes (15). Intra-arterial infusion of bleomycin was also used to treat head and neck cancers (16) and squamous cell carcinoma of the cervix (17), but the response rate for tumor regression was not superior to the results achieved by the iv route.

5-Fluorouracil (5-FU)

The pharmacokinetics of 5-FU and its metabolites have not been thoroughly analyzed due to the lack of sensitive and specific assay methods. Using a microbiologic assay, Clarkson et al (18) showed that after iv injection, the drug is very rapidly cleared from the patient's plasma; by 3 hours no detectable drug exists in the plasma. The total urinary excretion of the unchanged drug in patients with normal renal function is only 15.9% ± 6.8% of the administered dose. Recently, the 5-FU levels in human plasma after iv injection were studied using gas chromatography (19), isotope dilution-mass fragmentography (20), and high-pressure liquid chromatography (21). The results showed that the plasma elimination half-life for 5-FU is about 10–12 minutes and the concentrations drop below measurable levels at 1.5–2 hours after drug administration. The total plasma clearance of 5-FU was approximately 1300 ml/minute (21), which is greater than the normal hepatic plasma flow, and indicates other elimination routes in addition to liver clearance of the drug. Further studies showed that hepatic 5-FU extraction was > 90% (22) and was 25%–50% (23,24) during one liver passage. Systemic levels of 5-FU with hepatic arterial infusion were only 50%–80% of those with peripheral venous infusion (23,24). Therefore, treatment of liver cancers with hepatic arterial infusion of 5-FU should yield lower toxicity than in the case of iv infusion. The same study (24) also showed that drug level ratios in the liver after hepatic arterial versus peripheral venous infusion ranged from 1.4 to 1.5, suggesting that there are other elimination routes in addition to hepatic clearance of the drug and that hepatic arterial infusion may increase the response rate for anticancer treatments. For a target region with a small blood flow rate (eg, Q_T = 100 ml/minute), R_d = 22.67 and intra-arterial infusion should have great advantages over the iv route.

5-FU has been utilized for the treatment of head and neck cancer, and response rates of approximately 15% have been reported using a standard loading course (25). A higher response rate of about 45% has been observed when 5-FU is infused intra-arterially (16,26). A higher response rate using intra-hepatic arterial infusion of 5-FU to treat patients with progressive liver cancers has also been reported. Fifty-five percent of the evaluable patients among a total of 419 patients, three fourths of whom had had prior therapy with iv 5-FU and had been switched to the arterial infusion upon progression of disease, met the rigid criteria for improvement (27). This is considerably higher than the response rate of approximately 15%–20% for patients with colorectal cancer treated iv with 5-FU.

Melphalan

Melphalan is eliminated very rapidly from human patients, with a half-life of 1.44 ± 0.8 hours following iv bolus injection (28). The total body plasma clearance for a 70-kg patient averages 364 ± 204 (mean ± SD) ml/minute. However, the total 24-hour urinary excretion of the unchanged drug is only 13.0% ± 5.4% of the administered dose. The major mechanism of melphalan elimination in vivo appears to be rapid hydrolysis of the parent compound into mono- and di-hydroxymelphalan. Because of its very rapid disappearance from the body, melphalan infused intra-arterially should show superior antitumor effectiveness as compared with systemic routes of drug delivery (R_d = 7.07; Q_T = 100 ml/minute; hematocrit value = 0.4).

Clinical application of intra-arterial infusion with mel-

TABLE 1.—Therapeutic responses and toxicity from intra-arterial infusion of Adriamycin

Organ	Ref No.	No. of patients evaluable for response	No. of patients with—		Total No. of responses	No. of patients evaluable for toxicity	No. of patients with—							
			< 50% regression	> 50% regression			Stomatitis		Alopecia		Leukopenia		Cardiac toxicity	
Liver	5	9	2	1	3	10	5		5		1		2	(1)†
	7	11	—	—	3*	—	—		—		—		—	
	8	2	0	2	2	2	1		0		2		0	
	9	2	0	2	2	2	0		2		0		2	(2)†
	10	4	0	2	2	9	2		8		2		0	
Totals		28	2	7 (41%)	12 (43%)	23	8	(35%)	15	(65%)	5	(22%)	4	(17%) (3)†
Others	5	17	8	6	14	22	12		12		11		3	
	6	7	1	3	4	10	4		8		7		0	
	7	22	—	—	11*	—	—		—		—		—	
	8	9	0	5	5	—	—		—		—		—	
Totals		55	8	14 (42%)	34 (62%)	32	16	(50%)	20	(63%)	18	(56%)	3	(9%)

*Response defined as an objective reduction in tumor size and subjective regression of tumor as evidenced by resolution of pain and improvement in performance status.

†Probably died of cardiac toxicity.

phalan has been rare. Only one study with this technique (29) reported tumor regression in one of the three patients treated. Almost all localized treatment of malignant disease with melphalan has been by isolated regional perfusion, a method that results in very high melphalan concentrations in the perfused area with almost no systemic toxicity. Compared with conventional surgery, a 10-year study (30) of 202 patients with stage I malignant melanoma treated with isolation-perfusion of melphalan showed that the 2-, 5-, and 10-year determinate survival rates were 98%, 86%, and 83% respectively, while the corresponding rates for the control surgical group were 89%, 70%, and 57%.

Methotrexate

After iv injection, methotrexate disappearance from plasma is triphasic, with half-lives of 0.75, 2.0–3.5, and 10.5 hours respectively. The major route of elimination is renal clearance (31). Approximately 41% of the iv dose is excreted unchanged in the urine within 6 hours after administration, 90% within 24 hours, and 95% within 30 hours. The drug clearance from plasma is 110 ml/minute/m^2 under conditions of normal renal function. Therefore, intra-arterial injection of this drug should lead to higher tissue levels in the region supplied by the infusing artery compared with iv injection (R_d = 4.17; Q_T = 100 ml/minute; hematocrit value = 0.4; surface area = 1.73 m^2). Experimental data (32) confirmed this prediction of higher drug levels but the difference was small.

The slightly higher methotrexate levels after intra-arterial infusion may explain why the use of this technique has not shown an improved response rate (25). Comparison between intra-arterial infusion and systemic administration of methotrexate for the treatment of head and neck cancers shows that the overall response rates are not markedly different for the two forms of therapy (53% of 340 patients for intra-arterial treatment and 43% of 232 patients for systemic treatment), in spite of the fact that patients treated by the intra-arterial route had more localized disease. It should also be noted that the optimal systemic route of therapy for methotrexate produces an overall response rate of 56%, which is slightly higher than that for intra-arterial infusion of methotrexate (25).

In the treatment of cervical cancer with methotrexate, the response rate seems to be much better for the intra-arterial route but the toxicity is a major problem. Furthermore, the systemic methotrexate route has not received an adequate trial to confirm the superiority of intra-arterial infusion and to justify its higher expense and substantial risks (25).

DISCUSSION

Intra-arterial infusion is frequently used in the treatment of localized malignant disease. The hypotheses are: (*a*) that this technique can deliver a high concentration of the particular antitumor agent to localized cancers and hence produce a higher response rate than the systemic route of drug administration, and (*b*) that a significant amount of the drug will be removed after the first pass through the capillary bed of the target region and thus reduce the systemic drug availability and toxicity generated. With the utilization of linear pharmacokinetics, it can be shown theoretically that the drug levels in the regional tissues will be significantly increased provided

that the blood flow rate of the artery receiving the infusion is small and the drug is eliminated very quickly by the rest of the body. The systemic availability will be markedly reduced if a large amount of the drug is removed during the first pass through the infused region.

Experiments with intra-hepatic arterial infusion of 5-FU showed a significant reduction in systemic drug availability (22,23). However, the local tissue levels of Adriamycin and methotrexate infused intra-arterially were only slightly higher than those after iv injection (4,32). The small increase in the tissue concentration may be due to the large blood flow rate of the artery receiving the infusion.

It should be noted that comparisons of response rates using intra-arterial and iv routes of administration are often difficult to interpret because the drug combinations used and the diseases treated are often different. For example, responses occurred in nine of 16 patients for hepatic artery infusion of 5-fluoro-2′-deoxyuridine versus none of 18 patients for systemic routes of 5-FU, vinblastine, cyclophosphamide, etc, in the treatment of hepatoma (33). Furthermore, comparison of the response rates is usually between an intra-arterial infusion group with more localized disease and a systemic therapy group with localized and disseminated cancers. Even then, a review of five drugs, Adriamycin, bleomycin, 5-FU, melphalan, and methotrexate, infused intra-arterially as single agents indicates that only 5-FU, when it is infused intra-arterially, shows a higher response rate compared to iv treatment (26,27). The remaining drugs either have had inadequate trials or no significant differences existed in the response rates for the two forms of administration. Possible reasons for the lack of superiority for intra-arterial infusions are as follows: (*a*) there are small differences in the local tissue levels (however, according to equation [4], drug concentration can be increased by reducing the blood flow rate of the infusing artery); (*b*) the drug levels after both forms of administration are on the asymptotic portion of the dose-response curve where a large increase in drug levels results in a very small increase in the rate of response; and (*c*) there is a large variation in the response rates to a particular drug for different types of cancers.

Local toxicity is often a drawback of intra-arterial infusion therapy. However, there seems to be no significant reduction in the systemic toxicity in contrast to that which is expected. The reasons may well be the lack of controlled studies using large numbers of patients and the possibility of producing toxic metabolites after the drug passes through the infused target region.

From the present review of five anticancer agents, it appears that, except for 5-FU, there seems to be no justification for the higher expense and substantial risks of intra-arterial infusion therapy. A controlled, randomized study to further confirm the lack of difference in the response rates between systemic and arterial administration of these single agents in the treatment of each particular type of cancer is necessary.

APPENDIX
Drug Delivery of Intra-Arterial Versus Iv Injection

A pharmacokinetic model based on the physiology of the mammalian circulation is shown in figure 1 and will be used to study the behavior and characteristics of intra-arterial infusion. The drug is given by either iv or intra-arterial infusion into the body and is assumed to be eliminated by all the organs and tissues. The mechanisms of drug elimination are urinary excretion, metabolism by liver or hydrolysis in the body fluid, etc.

Since it has been shown (1) that R_d is independent of the rate of infusion, it is possible to have a very low infusion rate such that the integrals in equation [1] can be approximated by

$$\int_0^\infty C(t)dt \simeq C_\infty \Delta t \qquad \text{[A-1]}$$

where Δt is the interval of time that the concentration remains at the steady-state level. The approximation equation [A-1] improves as the infusion rate decreases because the parts of the area under the concentration curve prior to steady state and after the infusion is terminated will become negligible for the same quantity of drug administered. Therefore, equation [1] becomes

$$R_d = \frac{(C_i)_{ia}}{(C_i)_{iv}} \qquad \text{[A-2]}$$

at steady state. Although equation [A-2] is derived from an "infinite infusion steady state" model, it is appropriate to use for any rates and durations of drug infusion since it can be shown theoretically that the integration in equation [A-1] is independent of the rate and duration of the infusion as long as the total amount of drug administered is the same and as long as linear pharmacokinetics apply. This was also demonstrated by a computer simulation for intracarotid infusion of drug into brain (34).

The differential equations that describe the drug concentrations in the organs as functions of time are given as

$$V_P \frac{dC_P}{dt} = Q_P \left(\frac{C_L}{R_L} - C_P\right) - K_P C_P , \qquad [A-3]$$

$$V_L \frac{dC_L}{dt} = \sum_i Q_i \cdot \frac{C_i}{R_i} + Q_T \frac{C_T}{R_T} - Q_P \frac{C_L}{R_L} - K_L \frac{C_L}{R_L} + g_{iv} , \qquad [A-4]$$

$$V_i \frac{dC_i}{dt} = Q_i \left(C_P - \frac{C_i}{R_i}\right) - K_i \frac{C_i}{R_i} , \qquad [A-5]$$

and

$$V_T \frac{dC_T}{dt} = Q_T \left(D_P - \frac{C_T}{R_T}\right) - K_T \frac{C_T}{R_T} + g_{ia} \qquad [A-6]$$

where the subscripts P, L, T, and i denote the plasma, lung, target region (or tumor), and typical i-th compartment respectively. V is the volume of the tissue and Q is the compartmental blood flow rate. K is the intrinsic clearance of the drug which is the maximum capacity for drug elimination if there is no limit to the transport of the drug in that organ. The constant R is the equilibrium partition coefficient for the distribution of drug between the tissue and the venous blood. g_{iv} and g_{ia} are the drug injection functions which equal the rate of infusion k_o.

At steady state, all the differential terms vanish and the concentrations in each of the organs and tissues can be easily solved for both modes of drug administration. The results are as follows.

Iv Infusion

The concentrations in tissues are related to the plasma drug concentration by

$$C_L = \frac{(Q_P + K_P)}{Q_P} R_L C_P , \qquad [A-7]$$

$$C_i = \frac{Q_i R_i}{Q_i + K_i} C_P , \qquad [A-8]$$

and

$$C_T = \frac{Q_T R_T}{Q_T + K_T} C_P . \qquad [A-9]$$

The plasma concentration, in turn, is related to the rate of drug infusion by

$$C_P = \frac{k_o}{\frac{(Q_P + K_L) \cdot (Q_P + K_P)}{Q_P} - \sum_i \frac{Q_i^2}{Q_i + K_i} - \frac{Q_T^2}{Q_T + K_T}}$$

$$= \frac{k_o}{K_P + \frac{K_L (Q_P + K_P)}{Q_P} + \sum_i \frac{K_i Q_i}{Q_i + K_i} + \frac{Q_T K_T}{Q_T + K_T}} \qquad [A\text{-}10]$$

and

$$C_T = \frac{Q_T R_T}{Q_T + K_T} \cdot \frac{k_o}{K_P + \frac{K_L (Q_P + K_P)}{Q_P} + \sum_i \frac{Q_i K_i}{Q_i + K_i} + \frac{Q_T K_T}{Q_T + K_T}} \cdot \qquad [A\text{-}11]$$

Intra-Arterial Infusion

The relationship between the concentrations in plasma and tissues remains the same except for the tumor (or target) compartment whose concentration is

$$C_T = \frac{(Q_T C_P + k_o) R_T}{Q_T + K_T} . \qquad [A\text{-}12]$$

From the mass balance that input is equal to the amount eliminated,

$$k_o = K_P C_P + K_L \frac{C_L}{R_L} + \sum_i K_i \cdot \frac{C_i}{R_i} + K_T \frac{C_T}{R_T}, \qquad [A\text{-}13]$$

the relationship between the plasma concentration and the rate of infusion can be obtained. Thus,

$$C_P = \frac{k_o \left(1 - \frac{K_T}{Q_T + K_T}\right)}{K_P + \frac{K_L (Q_P + K_P)}{Q_P} + \sum_i \frac{Q_i K_i}{Q_i + K_i} + \frac{Q_T K_T}{Q_T + K_T}} \qquad [A\text{-}14]$$

and

$$C_T = \frac{R_T}{K_T + Q_T} \left(k_o + \frac{Q_T k_o \left(1 - \frac{K_T}{Q_T + K_T}\right)}{K_P + \frac{K_L (Q_P + K_P)}{Q_P} + \sum_i \frac{Q_i K_i}{Q_i + K_i} + \frac{Q_T K_T}{Q_T + K_T}}\right) . \qquad [A\text{-}15]$$

Effectiveness of Drug Delivery to the Tumor With Arterial Infusion

From equation [A-2], the advantage of drug delivery to the tumor through arterial infusion is given by

$$R_d = \frac{(C_T)_{ia}}{(C_T)_{iv}} \qquad [A\text{-}16]$$

at steady state. Therefore, from equations [A-11] and [A-15], equation [A-16] becomes

$$R_d = 1 + \frac{1}{Q_T}\left[K_P + \frac{K_L(Q_P + K_P)}{Q_P} + \sum_i \frac{Q_i K_i}{Q_i + K_i}\right], \qquad [A\text{-}17]$$

which is always greater than or equal to unity. The terms in the parentheses represent the sum of the apparent clearance of the drug from plasma, lung, and the rest of the body except for the target region.

Decreasing Drug Availability to the Systemic Circulation With Arterial Infusion

The advantage of reducing drug delivery and availability to the rest of the body by intra-arterial infusion is given by

$$R_d = \frac{(C_P)_{ia}}{(C_P)_{iv}} = \frac{(C_i)_{ia}}{(C_i)_{iv}} \qquad [A\text{-}18]$$

at steady state. From equations [A-10] and [A-14], it is simple to obtain

$$R_d = 1 - \frac{K_T}{Q_T + K_T}, \qquad [A\text{-}19]$$

where the last term represents the extraction ratio of the drug by the target region.

Nomenclature: C = drug concentration, K = intrinsic clearance, Q = blood flow rate, R = partition coefficient for the drug distribution between tissues, R_d = drug availability ratio of intra-arterial to iv infusion, V = volume of the compartment, k_0 = rate of infusion, t = time, δ = probability that a drug molecule will be lost when passed through an organ, and α = fraction of the cardiac output that flows through the infusion artery. Subscripts: L = lung, P = plasma, T = target organ or tumor, i = i-th compartment except for lung, plasma, and tumor, and s = systemic circulation.

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Methotrexate-Citrovorum Factor Used Alone and in Combination Chemotherapy for Advanced Hypernephromas [1]

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SUMMARY

Methotrexate-citrovorum factor (MTX-CF) therapy was administered to 20 patients with advanced renal cell carcinoma. Eight patients were treated with MTX-CF only. Two patients who received short-term rescue showed partial remissions along with severe toxicity including gastrointestinal symptoms and bone marrow depression. Twelve patients were treated with a combination of MTX-CF, vincristine, bleomycin, and an alkylating agent (either peptichemio or cyclophosphamide). In this latter group, patients with a glomerular filtration rate of > 70 ml/minute received 150–400 mg/m²/cycle of MTX. Patients with decreased renal function (< 70 ml/minute) received 35–100 mg/m²/cycle of MTX. Two of seven patients with a decreased glomerular filtration rate showed partial remissions and four patients had static disease. One patient in the group of five with normal renal function showed static disease. None showed objective improvement. The median survival time was 25 months for patients with partial remission and static disease and 4.75 months for patients with progression. This difference is highly significant ($P < 0.0005$). These results seem to justify further investigations of MTX-CF therapy in hypernephromas, even in the presence of impaired renal function.

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Renal cell carcinoma responds poorly to conventional chemotherapeutic agents (1,2). Talley (3) reported two responses in 91 patients treated with cytostatic agents.

The antitumor activity of aminopterin, a folic acid antagonist, was demonstrated > 30 years ago (4). Methotrexate (MTX), a less toxic analog, subsequently became standard treatment for acute lymphocytic leukemia and chorioendothelioma (5,6). The introduction of high-dose pulse infusions of MTX, with or without citrovorum factor (CF) rescue (7,8), led to the recent demonstration of significant efficacy of this agent in solid tumors previously considered resistant to antifols as well as to most other chemotherapeutic agents (9–14).

In a previous study we investigated the effect of using different dose schedules of MTX followed by CF rescue in various solid tumors, including eight hypernephromas. Two of the eight patients received CF rescue for only 48 hours. These two patients showed a therapeutic effect as well as severe toxicity despite normal renal function.

The lack of response in patients with hypernephroma receiving more extensive CF rescue, sufficient to prevent toxicity, led us to a trial of MTX-CF in combination with vincristine, bleomycin, and an alkylating agent (peptichemio or cyclophosphamide).

MATERIALS AND METHODS

MTX (Lederle Laboratories) was supplied in dry form in 50-mg vials. Fifty milligrams of MTX was dissolved in 10 ml of distilled water prior to use. CF (calcium leucovorin, Lederle Laboratories) was supplied in a solution of 3 mg/ml, in the form of 1-ml ampules.

MTX (250–750 mg/m²/treatment) was administered in

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TABLE 1.—Dose of MTX according to glomerular filtration rate

Cycle	Dose of MTX (mg/m²) for patients with glomerular filtration rate of—	
	> 70 ml/min	< 70 ml/min
1	150	35
2	200	75
3	250	100
4	300	No further increase
5	400	No further increase

a 1-hour infusion as single-agent therapy. For combination therapy, patients with normal renal function (glomerular filtration rate > 70 ml/minute) received 150 mg/m² of MTX as the first treatment, with the dose being escalated in subsequent cycles to 400 mg/m². Patients with decreased renal function (glomerular filtration rate < 70 ml/minute) received 35–100 mg/m² of MTX per treatment (table 1). It was presumed that patients with decreased renal function would have abnormal rates of MTX decay, therefore predisposing these patients to MTX toxicity.

The first three infusions of MTX were given at 10-day intervals. Subsequently, MTX was administered every 3 weeks. Patients were hospitalized 3 days for each treatment.

CF rescue was started 3 hours following the infusion of MTX and consisted of 3 mg of CF given im every 3 hours for the first 24 hours, followed by 3 mg every 6 hours for the second 24 hours and 3 mg every 3 hours during the third 24 hours, whenever extended rescue was desired.

Hydration was carried out with iv infusions of 2500 ml of 5% dextrose or oral fluid intake of at least 2500 ml/day. Alkalinization consisted of 20 mEq of sodium bicarbonate given iv every 8 hours, starting immediately prior to MTX.

Figure 1 illustrates the therapeutic schedule used for combination therapy. Vincristine (0.4 mg/m²), bleomycin (10 mg/m²), and cyclophosphamide (300 mg/m²) were all given by 10-minute infusion mixed with the MTX. This 10-minute infusion of MTX in combination therapy was chosen to obtain a higher peak plasma level and perhaps also a higher tumor uptake of MTX. In seven patients another alkylating agent, peptichemio (25 mg/m²), was substituted for the cyclophosphamide. Peptichemio[7] (15) was diluted in 250 ml of dextrose and given iv after the other drugs. The dose of cyclophosphamide was reduced to 150 mg/m² when leukocyte counts were < 3.0×10^9/liter. Bleomycin was discontinued after reaching a total dose of 300 mg.

Because significant intratumor fibrin deposits have been demonstrated in renal adenocarcinomas, anticoagulation with heparin was performed in eight patients without hematuria, who received combination therapy, with the aim of enhancing the effect of cytotoxic therapy (16). Patients received heparin (12,000 units) in depot form every 12 hours for a total of six doses during the chemotherapeutic cycle.

Objective response was defined as complete response when there was disappearance of all measurable lesions. Partial response (PR) was defined as a > 50% reduction in the product of the perpendicular diameters of one or more measurable lesions without progression in any other lesions. Progressive disease (PD) was defined as a > 25% increase in the product of the perpendicular diameters. A decrease of < 50% or an increase of < 25% was defined as static disease (SD).

Changes in tumor size were determined by comparison of measurements taken before therapy and at the time of maximal effect. The duration of remission was measured from the time of the first objective signs of regression until PD. The statistical significance of differences in median survival time was determined by the log-rank test.

A total of 20 patients with histologically proven hypernephromas were studied. All had recurrent or inoperable disease. Prior therapy, when present, consisted of surgery and/or radiotherapy. Most frequently metastases were in the lung and bones. All patients had creatinine clearance tests prior to entering the study. Their glomerular filtration rate ranged from 8 to 112 ml/minute. The Karnofsky status was > 50% prior to the first treatment.

RESULTS

Methotrexate-Citrovorum Factor as Single-Agent Therapy

Eight of the above patients, four females and four males, 39–66 years old, were treated with MTX-CF alone. All had recurrent, metastatic, or inoperable hypernephromas. Three patients had had radical surgery 10–12 months previously. They entered the study as they were developing distant metastases. One patient had had palliative resection of the primary tumor. Three patients had had radiation therapy only and one patient had had no previous treatment.

All had normal renal function. Each received at least three cycles of MTX-CF as described in the Materials and Methods section.

Six of the eight patients received the 3-day course of CF described above with each treatment. None showed

[7]Peptichemio is a mixture of 6 peptides of *m*-(di(2-chloroethyl)amino)-L-phenylalanine. It was supplied by the Instituto Siero Terapico Milanese, Milan, Italy, through the European Organization for Research on Treatment of Cancer Screening Group.

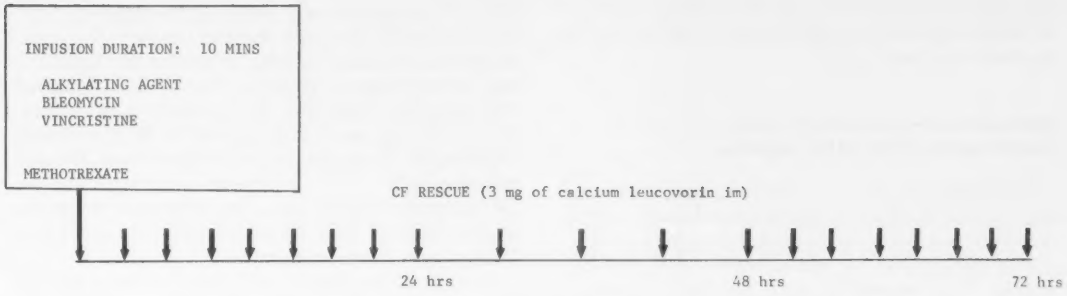

FIGURE 1.—Schedule of combination therapy.

any severe toxic reactions or any tumor effect. Their diseases progressed and they died 1–10 months following diagnosis.

The remaining two patients received a shortened CF rescue cycle for the first 48 hours only (fig 2). The primary tumor was not resectable in either patient. Both had received prior radiotherapy. Patient 1 (a 65-year-old male) developed lung metastasis during radiation therapy. Patient 2 (a 60-year-old female) showed a large liver metastasis 5 months after irradiation. Both received three cycles of MTX starting at 250 mg/m^2 in the first cycle and escalated to 500 mg/m^2 in the second cycle and to 750 mg/m^2 in the third cycle. The CF rescue started 2 hours after the end of the infusion. Three mg of CF was given im every 3 hours during the first day and every 6 hours during the second day (fig 2). The total dose of CF rescue was 36 mg.

After the third treatment both patients developed severe toxic reactions, including stomatitis, severe diarrhea, and bone marrow depression. Peripheral blood cell counts dropped in patient 1: the wbc count was 0.3×10^9/liter (differential counts were not available), the hemoglobin level was 3.4 mmols/liter, and the platelet count was 50×10^9/liter. In patient 2 the lowest counts were as follows: wbc count, 3.2×10^9/liter (44 neutrophils, 23 eosinophils, 30 lymphocytes, and 3 monocytes); hemoglobin level, 5.9 mmols/liter; and platelet count, 76×10^9/liter. Both patients developed eosinophilia during therapy.

Eosinophilia was present in patient 1 until relapse occurred and in patient 2 only during the toxic phase after MTX therapy. Both patients recovered without receiving granulocyte or platelet transfusions.

Both patients achieved a PR. In patient 1 the first signs of regression of lung metastasis were visible on roentgenogram 3 weeks after starting therapy. Maximal regression was achieved after 6 weeks. The inoperable primary tumor also regressed slightly. The regression lasted 4 months without maintenance therapy. The survival time was 8 months.

In patient 2 the size of the primary tumor (10×11 cm) and the liver metastasis (10×10 cm) was determined by angiography before starting the MTX-CF therapy. Before therapy the liver was palpable 10 cm below the costal margin. After 4 weeks the liver began to shrink and it was no longer clinically enlarged after 16 weeks. The regression was confirmed angiographically. The liver metastasis disappeared almost completely and the pri-

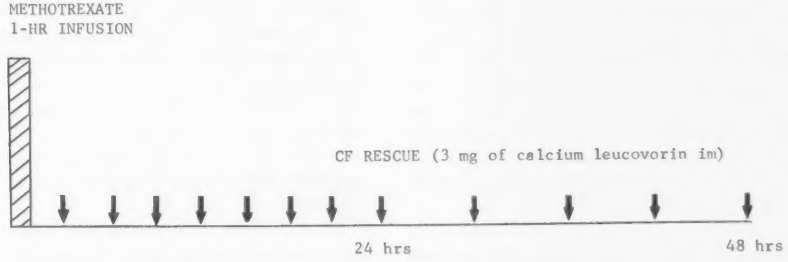

FIGURE 2.—Schedule of MTX single-agent therapy with shortened CF rescue (48 hrs).

mary tumor shrank to 8 × 7 cm. The patient is still alive 44 months later without maintenance therapy and no PD has been observed to date.

Methotrexate-Citrovorum Factor in Combination With Other Agents

Twelve patients, six males and six females, 15–77 years old, received MTX-CF in combination therapy. All had recurrent, metastatic, or inoperable hypernephromas.

Four patients had had radical surgery 2–8 years previously. They entered the study because of metastasis (three patients) or local recurrence (one patient). Five patients had had palliative resection of the primary tumor. Two of them had had irradiation therapy. In one patient occlusion of the renal artery preceded surgery. One patient had had radiotherapy only. Two patients had received no previous treatment.

The doses, schedule of combination therapy, hydration, and alkalinization are described in the Materials and Methods section. MTX doses were adjusted according to renal function (table 1). All patients received the 3-day course of CF described above (fig 1).

The total dose of CF was 60 mg/cycle regardless of the MTX dose. Three patients with osteolytic bone lesions received palliative radiation therapy in addition to the chemotherapy.

Three patients developed moderate stomatitis. One of these three had a decreased glomerular filtration rate prior to treatment. Therapy continued without delay, however. One patient showed elevation of liver enzymes after therapy. No bone marrow depression was observed. Two patients showed dermatitis due to bleomycin. In both, the drug was discontinued. Significant eosinophilia (absolute eosinophil counts of 0.5–1.27 × 10^9/liter) was observed in four patients, two showing PR and two showing SD. Eosinophilia lasted as long as no progression occurred.

Five patients had PD and five had SD lasting 5–20 months. Two patients achieved PR. The first of these two patients was a 67-year-old male, who had undergone radical surgery 35 months before starting therapy because of lung and skin metastasis. The skin metastasis, measuring 3 × 5 cm, disappeared completely after 3 months; the regression was confirmed histologically. The lung metastasis disappeared almost completely. The regression lasted for 5 months. During this time the patient was regularly treated with MTX-CF combination therapy. He later refused further treatment; after 3 months he developed bone metastasis and he died 10 months after the start of treatment.

The second patient who achieved a PR, a 70-year-old female, had an inoperable primary tumor and received therapy immediately after diagnosis had been confirmed. The size of the primary tumor (12 × 9 cm) was determined by angiography and ultrasonic investigation. After 12 months of therapy the tumor had shrunk to 7 × 5 cm. After 13 months skin and bone metastases developed and the patient became immobile. Therapy was combined with palliative irradiation. After completion of the irradiation, therapy was continued with the MTX increased to 500 mg/m². There was a second improvement. The skin metastasis (10 × 10 cm) regressed completely and the patient became mobile again. She is still alive 26 months after the start of MTX-CF combination therapy without further signs of progression.

Analysis of the results with regard to renal function shows that two of seven patients with a glomerular filtration rate of < 70 ml/minute receiving MTX in low doses (35–100 mg/m²) achieved PR, while four showed SD and one had PD. In the group of five patients with a glomerular filtration rate of > 70 ml/minute, who received MTX in doses between 150 and 400 mg/m², four patients showed PD, with the dose of MTX being increased to 750 mg/m² in one of them; only one patient in this category had SD (table 2).

Five of the 12 patients are still alive. The observation time is now 5–26 months from the start of treatment. The patients have received six to 33 treatments. Four patients are still receiving therapy.

All patients who had PD have died. The median survival time in this group was 5.25 months. The median survival time of all other patients (PR + SD) was 24.6 months. This difference is statistically significant ($P < 0.05$).

Of the eight patients who received heparin in addition to the chemotherapy, two had PR, three had SD, and three had PD.

DISCUSSION

Kidney cancer has proven to be resistant to chemotherapy (1–3,17,18). A low response rate has been reported only with hormonal treatment (19–21).

Used alone in our series, MTX with 48-hour CF rescue was effective but very toxic. MTX followed by 72-hour CF rescue in patients with normal renal function was ineffective in this series. In the group of patients treated with combination therapy the patients with a decreased glomerular filtration rate showed substantial tumor regressions. These results were achieved with MTX doses as low as 35–100 mg/m² followed by CF rescue (60 mg/cycle). The delayed excretion of MTX might be responsible for the tumor response in the patients with impaired renal function, despite the low doses of MTX administered (22,23).

Because of the above and the fact that vincristine, bleomycin, and alkylating agents are not effective when used alone (2,24), it is most likely that the MTX was the effective drug in the combination we used.

TABLE 2.—Characteristics of 12 patients with advanced hypernephromas treated with combination therapy

Patient No.	Sex/age (yrs)	Mos from diagnosis to start of therapy	Recurrence or primary*	Location of metastasis	Glomerular filtration rate (ml/min)	Creatinine level (mg/100 ml)	No. of treatments	Maximum dose of MTX (mg/m²)	Tumor response	Duration of response (mos)	Side effects	Survival (mos)
1	M/67	—	+	—	8	1.8	17	100	SD	12	—	13‡
2	M/70	1	+	Lung	35	1.5	17	100	SD	11	Dermatitis, eosinophilia	15‡
3	F/70	—	+	Lung, bones	35	1.2	33	100	SD	20	—	23‡
4	M/67	36	–	Lung, skin	45	1.4	10	100	PR	5	Eosinophilia	10§
5	F/70	—	+	Bones,† skin	45	1.0	33	100, 500†	PR	12, 10†	Eosinophilia	26‡
6	F/52	2	–	Bones	61	1.0	30	100	SD	6	—	25§
7	M/55	12	+	Lung	65	1.2	3	100	PD	—	—	4§
8	M/47	12	+	Lung, liver	76	1.2	11	400	PD	—	Stomatitis	10§
9	F/15	2	+	—	76	0.8	6	400	SD	4	Increased SGOT, increased SGPT	5‡
10	F/77	24	+	—	77	1.2	9	400	PD	—	—	7§
11	M/52	24	–	Lung	81	1.0	6	400	PD	—	Stomatitis	5§
12	F/56	96	+	Bones	111	0.8	5	750	PD	—	Eosinophilia	5§

*+ = patient was being treated for recurrent or inoperable disease; – = patient was being treated for metastatic disease.
†Patient had metastasis after 13 mos and improved a 2nd time after irradiation and increase in MTX dose.
‡Patient still alive.
§Patient died.

Hypernephromas in advanced stages generally have a poor prognosis (17,18,25), although occasionally spontaneous remissions are reported (26,27). According to Bloom (19) these are rare events. It seems remarkable that among 20 patients, four PRs and five SDs were achieved, resulting in an improved quality of life and a prolonged survival time. The median survival time of the nine patients with PR or SD was 25 months, while it was 4.75 months in the patients with PD. The difference is highly significant ($P < 0.0005$). Survival time was also prolonged compared with published information (3,17, 18,21). An unexpected observation during this study was the effect on the eosinophil counts. An elevation of eosinophils in patients responding to MTX-CF treatment was subsequently observed in other patients with various solid tumors treated in this manner (unpublished results).

MTX seems to be an effective agent in advanced hypernephromas. Toxicity did not occur in the low dose range we used in the patients with a decreased glomerular filtration rate. The pharmacokinetics of low doses of MTX followed by CF rescue, in patients with impaired renal function, had not been studied until now (28–32). Such studies may be of value to clarify effective means for the use of MTX and CF rescue.

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Adriamycin Given as a Weekly Schedule Without a Loading Course: Clinically Effective With Reduced Incidence of Cardiotoxicity [1,2,3]

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SUMMARY

Three hundred and thirty-six patients with a variety of tumors were treated with Adriamycin given weekly as an iv bolus of 1 mg/kg with subsequent doses adjusted for hematologic toxicity. This weekly schedule, not utilizing a loading course, resulted in only a 20% incidence of stomatitis. The number of evaluable patients and the percent with objective responses (respectively) according to tumor type were: lung (57 patients, 14%); sarcoma (62, 24%); breast (31, 35%); transitional cell carcinoma (17, 29%); non-Hodgkin's lymphoma (17, 29%); head and neck (16, 13%); colorectal (13, 0); ovarian (eight, 25%); and other (53, 11%). These response frequencies are comparable to those reported for every-3-week regimens using 60–75 mg/m^2 of Adriamycin. Sixteen patients were given 450–550 mg/m^2 of Adriamycin, five were given 550–600 mg/m^2, and ten were given > 600 mg/m^2. None of the study patients developed definite evidence of drug-induced congestive heart failure. Therefore, Adriamycin given as a weekly schedule provides a clinically effective alternative to the every-3-week schedule of administration. The weekly schedule is associated with tolerable toxicity and a decreased risk of developing drug-induced congestive heart failure.

[Cancer Treat Rep 64:47–51, 1980]

Adriamycin, one of the anthracycline antibiotics, is one of the most useful antitumor agents currently available (1). Unfortunately, its administration is limited both by acute reversible toxicity and by irreversible cardiomyopathy which appear to be related to the cumulative dose of Adriamycin given. The risk of cardiomyopathy has been reported to be < 1% in patients receiving a total dose of < 550 mg/m^2 but > 30% in patients receiving a total dose of > 600 mg/m^2 (2,3). It appears that the various toxic effects of Adriamycin are dependent on the schedule of administration. Bonadonna et al (4) noted stomatitis in 78% of patients treated with weekly Adriamycin after a

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[4]The following members of the Western Cancer Study Group participated in this study: *J. R. Bateman*, *W. S. Paroly*, and *R. T. Chlebowski* (Los Angeles County-University of Southern California Medical Center); *J. Block* (Harbor General Hospital, Torrance, CA); *R. Bohannon* (San Francisco General Hospital, CA); *J. Brook* (Long Beach Veterans' Administration Hospital, CA); *F. Bull* (University of Michigan, Ann Arbor); *M. Corder* (Letterman General Hospital, San Francisco, CA); *A. Glass* (Kaiser Permanente Hospital, Portland, OR); *T. Godfrey* (Loma Linda University, CA); *C. Hendrickson* (Kaiser Permanente Hospital, Oakland, CA); *J. Hueser* and *R. P. Pugh* (Ellis Fischel State Cancer Hospital, Columbia, MO); *E. M. Jacobs* (University of California, San Francisco); *C. Kardinal* (Oakland Naval Hospital, CA); *R. Mass* (Portland Veterans' Administration Hospital, OR); *J. McCracken* (Madigan Army Medical Center, Tacoma, WA); *R. Reynolds* (David Grant Medical Center, Travis Air Force Base, CA); *H. Rosensteil* (Fresno Veterans' Administration Hospital, CA); *L. Sadoff* (Kaiser Permanente Hospital, Los Angeles, CA); *I. Silverberg* (Mt. Zion Hospital, San Francisco, CA); *J. Solomon* (City of Hope Medical Center, Duarte, CA); *W. Wilson* (Western Montana Clinic, Missoula, MT); and *T. F. Pajak* (*present address*: Cancer and Leukemia Group B, Scarsdale, NY [previously Statistician for the Western Cancer Study Group]).

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TABLE 1.—Definition of toxicity criteria

Grade	Leukopenia (cells/mm³)	Thrombocytopenia (cells/mm³)	Nausea and/or vomiting	Stomatitis and/or diarrhea	Alopecia
1	3000–3999	100,000–124,999	Mild	Mild	Mild
2	2000–2999	60,000–99,999	Moderate	Moderate	Moderate
3	1000–1999	35,000–59,999	Severe	Severe (unable to swallow)	Severe (total)
4	<1000	<35,000	Life-threatening (bleeding or electrolyte imbalance)	Life-threatening	

loading dose was given. However, only 23% of patients developed this complication when the drug was given as a single bolus every 3 weeks. The incidence of moderate or severe bone marrow depression also dropped from > 60% to 45% with the every-3-week schedule. Recently, Weiss et al (5) reported results of treatment of 442 patients receiving the original weekly schedule as described by Bonadonna et al (4). In this series, not a single instance of cardiomyopathy was observed, even though 50 patients received > 600 mg/m² of Adriamycin.

In 1971, the Western Cancer Study Group began a phase I–II study of Adriamycin utilizing a schedule of weekly administration. No loading dose was employed; rather, a high initial dose of 1 mg/kg was given, with reduction to 0.5 mg/kg when myelosuppression occurred. The purpose of this paper is to report the response and toxicity of Adriamycin administration occurring among the 336 patients treated with this protocol.

MATERIAL AND METHODS

Patients who were entered in the study had a variety of neoplasms. All patients had measurable disease parameters. No minimal performance status or life expectancy was required. Pretreatment evaluation included cbc, BUN, liver function tests, ECG, and chest roentgenogram. There were no minimum laboratory requirements for entry in the study, and a history of cardiac disease did not exclude patients from the study.

Treatment was initiated at a dose of 1 mg/kg. With the onset of hematologic toxicity (wbcs < 4000/mm³ or platelets < 100,000/mm³), the dose was reduced to 0.5 mg/kg and was continued at that level. Responses were classified as either complete response, defined as the total disappearance of all measurable lesions with the appearance of no new lesions for ≥ 1 month, or partial response, defined as a reduction in tumor by ≥ 50% of the cross-sectional area of all measurable lesions, with the appearance of no new lesions for ≥ 1 month. Minor change, no change, or progression of any measurable lesions during therapy was considered as no objective response. In the event of stabilization of disease without actual objective tumor regression, Adriamycin was continued until disease progression occurred. Before discontinuing the study for disease progression, a total dose of at least 4 mg/kg of Adriamycin was given. A cumulative dose of Adriamycin not exceeding 20 mg/kg (approximately 700 mg/m²) was recommended for the protocol.

Patients were evaluable for toxicity after one dose of Adriamycin was given. Criteria used in grading toxicity appear in table 1. Patients were considered evaluable for response if they received the equivalent of a single bolus dose (60 mg/m²) and survived for at least 21 days.

RESULTS

Three hundred and thirty-six patients were entered in the study. All patients were evaluable for toxicity and 274 were evaluable for response. Reasons for exclusion from the study included no measurable parameters (one patient), < 60 mg/m² of Adriamycin received (30), and death < 21 days after entering the study (31). Most of the excluded patients died within 21 days after entering the study. One half of these unevaluable patients had Karnofsky performance scores of < 60 and required hospitalization prior to the administration of Adriamycin. One hundred and sixty of the patients evaluable for response had received prior chemotherapy.

All patients initially received 1 mg/kg (approximately 35 mg/m²) of Adriamycin. However, 215 of the 336 patients (64%) subsequently required dose reduction to 0.5 mg/kg because of hematologic depression. Furthermore, 89 of the remaining 121 patients who received full-dose treatment had fewer than four courses of Adriamycin. Few patients tolerated long-term treatment with 1 mg/kg; only four patients received > 12 courses without dose reduction.

The toxicity observed with weekly Adriamycin is summarized in table 2. Only 9% of all patients experienced no toxic effects. However, of the 31 patients in this group, 16 died of their disease within 14 days and two others received < 60 mg/m² of Adriamycin. If it is to be assumed that a 14-day followup is reasonable for toxicity to become

TABLE 2.—Toxicity data for 336 patients receiving weekly Adriamycin

Toxic effect	% of patients with toxicity grade— 1	2	3	4	Total %
Leukopenia	23	28	17	7	75
Thrombocytopenia	11	7	17	0.5	36
Nausea and/or vomiting	26	18	6	3	53
Stomatitis	10	7	3	0.2	20
Diarrhea	2	1	0	0	3
Alopecia	13	19	23	—	55

apparent, then only 13 patients (4%) in the entire treatment group were free of toxicity.

Leukopenia was severe, and seven patients died during periods of leukopenia with infectious complications. Within this group, three patients received full-dose Adriamycin in the presence of elevated total bilirubin. Therefore, four patients (1.2%) unpredictably developed fatal complications from therapy. In three of these four patients, the nadir of leukopenia occurred between Days 10 and 14 of treatment, suggesting that the initial dose of 1 mg/kg was responsible. Overall, leukopenia (wbcs $< 4000/mm^3$) was seen in 75% of the patients.

Thrombocytopenia was less frequent and less severe than leukopenia. Serious bleeding secondary to thrombocytopenia did not occur. Two patients developed upper gastrointestinal bleeding, but neither stomatitis nor thrombocytopenia was present. The etiology in both cases was felt to be gastritis, with nausea and vomiting from the drug being a contributing factor.

Gastrointestinal toxicity, most frequently manifested as nausea and vomiting, was seen in moderate severity in 27% of patients. The incidence of stomatitis was relatively rare (20%), with only one patient developing severe stomatitis with ulceration and secondary infection.

Thirty-one patients received > 450 mg/m^2 of Adriamycin. Of these 31 patients, eight received between 450 and 500 mg/m^2, eight received between 500 and 550 mg/m^2, five received between 550 and 600 mg/m^2, and ten received between 600 and 1000 mg/m^2. Twenty-one of these 31 patients at high risk of cardiomyopathy received at least 1 month of followup (mean, 12 weeks). In this group, ECG abnormalities included non-specific ST-T wave changes in ten patients and premature ventricular contractions in four. Cardiac failure as the primary cause of death was not observed in any of the 336 patients receiving weekly Adriamycin therapy, including the 31 who received a dose of > 450 mg/m^2.

Three patients receiving < 450 mg/m^2 of Adriamycin had signs or symptoms consistent with congestive heart failure (CHF), but review of these charts demonstrated that factors other than Adriamycin were probably responsible for the clinical findings. One patient developed ECG changes consistent with an acute myocardial infarction during therapy but had no cardiac symptoms at any time and died of tumor progression. Another patient developed shortness of breath and atrial fibrillation in association with a pulmonary embolus; the symptoms resolved with digitalization, and the patient died of tumor progression. At the time of death, no CHF or cardiomegaly was present. The third patient developed atrial flutter 3 weeks after being removed from the study, had successful cardioversion, and had no evidence of cardiomegaly, pulmonary congestion, or cardiomyopathy at postmortem examination.

Responses for the entire group of patients are summarized in table 3, and responses for patients with sarcoma and lung cancer are summarized in tables 4 and 5, respectively. These tables also include the response frequency calculated to include all patients entered in each disease type. Objective responses were seen in 35% of evaluable patients with breast cancer, in 29% of those with non-Hodgkin's lymphoma, in 29% of those with transitional cell cancer, and in 24% of those with sarcoma. Median durations of response included 4.2 months in patients with breast cancer, 6.3 months in patients with non-Hodgkin's lymphoma, and 5.4 months in patients with sarcoma.

DISCUSSION

Long-term administration of Adriamycin on an every-3-week schedule is limited by dose restrictions recommended to minimize the risk of cardiomyopathy (2,3). Furthermore, no currently available, noninvasive methodology exists which can reliably predict this complication in a clinically useful manner (6,7). Recently, however, Weiss et al (5) reported the Central Oncology Group (COG) experience with Adriamycin given as a short-loading course followed by weekly administration at a dose of 0.4 mg/kg. In that study, no patients developed Adriamycin-induced CHF, including 50 patients who had received > 600 mg/m^2. This was a startling result since previous experience with the every-21-day schedule had suggested that patients receiving > 600 mg/m^2 of Adriamycin would have about a 30% chance of developing drug-induced CHF (2,3).

TABLE 3.—Response data for 336 patients receiving weekly Adriamycin

Diagnosis	Total No. of patients— Entered	Total No. of patients— Evaluable	No. of responses Partial	No. of responses Complete	% objective response based on— Evaluable patients	% objective response based on— All patients entered
Lung cancer	74	57	7	1	14	11
Sarcoma	69	62	10	5	24	22
Breast cancer	41	31	6	5	35	27
Transitional cell carcinoma*	23	17	0	5	29	22
Lymphoma	22	17	3	2	29	23
Head and neck cancer	18	16	2	0	13	11
Colorectal carcinoma	15	13	0	0	0	0
Ovarian carcinoma	13	8	2	0	25	15
Miscellaneous†	61	53	3	3	11	10
Total	336	274	33	21	20	16

*Includes bladder (18 patients), renal pelvis (3), and ureter (2).

†Includes testicular (8 patients), cervix (5), prostate (4), thyroid (4), kidney (2), adrenal (2), esophagus (2), neuroblastoma (2), unknown primary carcinoma (15), and 1 patient each with thymoma, mesothelioma, cardiac mesenchymoma, chordoma, retroperitoneal choriocarcinoma, mediastinal teratocarcinoma, skin carcinoma, pancreatic carcinoma, Wilms' tumor, müllerian duct carcinoma, uterine carcinoma, male breast cancer, and unclassifiable tumor.

TABLE 4.—Response data for 69 patients with sarcoma receiving weekly Adriamycin

Diagnosis	Total No. of patients— Entered	Total No. of patients— Evaluable	No. of responses Partial	No. of responses Complete	% objective response based on— Evaluable patients	% objective response based on— All patients entered
Osteogenic sarcoma	21	20	3	0	15	14
Leiomyosarcoma	12	11	3	2	45	42
Fibrosarcoma	6	6	1	1	33	33
Other soft tissue sarcomas	19	18	2	0	11	11
Ewing's	2	1	0	1	100	50
Kaposi's	1	1	0	0	0	0
Unspecified	8	6	1	1	33	25
Total	69	63	10	5	24	22

In the present study, no instance of Adriamycin-induced CHF was seen in the 336 patients receiving treatment with a weekly Adriamycin regimen. Thirty-one of these patients received > 450 mg/m^2 of Adriamycin. These data support the schedule-dependence of Adriamycin-induced CHF. These patients, along with the 442 patients from the COG trial (5), are included in a current review by Von Hoff et al (8) of > 4000 patients who received Adriamycin at various treatment schedules. That retrospective analysis confirmed that a weekly dose schedule of Adriamycin was associated with a significantly lower incidence of CHF compared to the usual every-21-day regimen. A lower than expected incidence of CHF was also reported using weekly Adriamycin in combination with other anticancer agents (9). The mechanism of this schedule-dependent cardiac toxicity has not been defined. However, Adriamycin administration on a weekly schedule in lower doses results in greatly reduced peak plasma levels of drug compared to every-3-week schedules (10). As a result, exposure of heart tissue to Adriamycin may

TABLE 5.—Response data for 74 patients with lung cancer receiving weekly Adriamycin

Diagnosis	Total No. of patients— Entered	Total No. of patients— Evaluable	No. of responses Partial	No. of responses Complete	% objective response based on— Evaluable patients	% objective response based on— All patients entered
Squamous cell	23	19	2	0	11	9
Adenocarcinoma	16	11	1	0	9	6
Oat (small) cell carcinoma	11	6	0	1	17	9
Large cell carcinoma	1	0	0	0	0	0
Unspecified	23	21	4	0	19	17
Total	74	57	7	1	14	11

be below the threshold level required for myocyte injury (11).

The initial dose of Adriamycin used in this study was greater than that used in the COG trial (1 versus 0.4 mg/kg/week). However, 64% of the patients required a dose adjustment to 0.5 mg/kg (approximately 15–20 mg/m^2) because of hematologic toxicity, and long-term treatment with 1 mg/kg was tolerated by only four patients. Analysis of the drug-associated deaths demonstrated that in three of four cases, the nadir wbc count associated with sepsis and death occurred within 14 days after the initiation of treatment. Therefore, a lower initial dose of Adriamycin of 15–20 mg/m^2/week could be recommended for a clinical trial involving a weekly schedule of administration.

Previous reports using weekly Adriamycin have emphasized the high incidence of stomatitis as one of the limiting toxic effects of such a regimen. However, in the current trial, only 20% of patients experienced stomatitis with only one of 336 patients developing marked ulceration and secondary infection. In reports associated with frequent stomatitis (4,5), Adriamycin therapy was initiated with a loading course on Days 1–3 and 8–10 before the weekly schedule was begun. This suggests that the loading course, rather than the weekly schedule, may be responsible for most of the stomatitis.

In this report, the frequency of objective response to weekly Adriamycin administration was quite high, especially considering the extensive prior treatment received by these patients. The responses of patients evaluable for response with breast cancer (35%), sarcoma (24%), ovarian cancer (25%), lymphoma (29%), and lung primaries (14%) are all comparable to those reported for every-3-week regimens using 60–75 mg/m^2 of Adriamycin (12–14). Therefore, Adriamycin given as a weekly schedule appears to provide a clinically effective alternative to the every-3-week schedule of administration. The weekly schedule is associated with tolerable toxicity and a decreased risk of Adriamycin-induced CHF.

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Phase I Evaluation of 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) by a Weekly Iv Dose Schedule [1,2,3]

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SUMMARY

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA), a derivative of acridine, was evaluated in patients with advanced malignant neoplasms in a phase I clinical trial. The drug was administered in an iv infusion on a weekly × 4 schedule. Seventy-four courses of AMSA were administered to 34 patients over a dose range of 20–90 mg/m^2. Dose-limiting toxicity, which was solely hematologic, was observed at 90 mg/m^2. Objective antitumor effects were observed in two of 22 evaluable patients: a partial remission in a patient with abdominal desmoid fibrosarcomas and a minor regression in a patient with adenocarcinoma of the colon. A dose of 70–90 mg/m^2 would be suitable for trials of AMSA on a weekly basis.

[Cancer Treat Rep 64:53–55, 1980]

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) is an acridine derivative synthesized by Cain et al (1). It has been selected for clinical trial because of its impressive antitumor activity in a wide range of transplanted murine tumors, including B16 melanoma, L1210 and P388 leukemias, CD breast tumor, and Lewis lung tumor (2). AMSA produces its cytotoxic effect by interaction with DNA that is based upon intercalation and external binding (3,4). Shoemaker et al (5) and Cysyk et al (6) established a correlation between the therapeutic activity of AMSA in B16 melanoma and the drug's capacity to localize within the tumor cells; the subcellular localization was within the melanin-containing cytoplasmic granules.

In rodents, Cysyk and colleagues (6,7) also found localization of activity initially in the kidney, spleen, and liver, with prolonged retention in the liver. The radioactivity was present primarily as metabolites of AMSA. Degradation was largely mediated by nonenzymic nucleophilic attack by endogenous thiols (glutathione) to form a thioether; the metabolites were subsequently excreted in the bile. Preclinical toxicology studies in dogs and monkeys demonstrated myelosuppression and injury to the kidneys and liver.[6] Investigators at other centers, using AMSA in a daily × 3 schedule (8) or as a single large dose at 28-day intervals (9), have reported the drug's principal toxic effects to be hematopoietic suppression and local phlebitis. This communication reports a phase I evaluation of AMSA given by weekly administration for four doses or to toxicity, with courses repeated at 6-week intervals.

PATIENTS AND METHODS

Thirty-four patients with histologically proven advanced neoplasms, 31 with solid tumors and three with

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[3]AMSA was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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[5]We are grateful to Wendy Werner for her assistance in the preparation of this manuscript.

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[6]Clinical brochure: *m*-AMSA, methanesulfon-*m*-anisidide, 4′-(9-acridinylamino) NSC-249992. Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, Aug 1977, 28 pp.

TABLE 1.—Patient characteristics

No. of patients in study	34
No. of men/women	21/13
Median age in yrs (range)	54 (22–71)
No. of patients with—	
Prior chemotherapy	30
Prior radiation therapy	14
No prior therapy	1
Median performance status (range)	60 (30–80)

TABLE 2.—Diagnostic characteristics

Diagnosis	No. of patients
Lung carcinoma (non-oat cell)	5
Colonic carcinoma	6
Lymphocytic lymphoma	1
Hodgkin's disease	2
Testicular carcinoma	7
Ovarian carcinoma	2
Cervical carcinoma	1
Breast carcinoma	2
Hepatoma	2
Renal carcinoma	1
Sarcoma	2
Salivary gland carcinoma	1
Adenocarcinoma, unknown primary	2

lymphomas, were entered in the initial trial of AMSA at the Memorial Sloan-Kettering Cancer Center (New York, NY) (tables 1 and 2). Pretreatment evaluation included full medical history and physical examination, assessment of performance status (Karnofsky scale), weight, cbc with differential and platelet count, SMA 6 and 12 serum biochemistry profiles as well as 5′-nucleotidase, electrocardiogram, chest roentgenogram, urinalysis, and radionucleotide scans when indicated by clinical assessment. The first five patients started treatment as inpatients; thereafter, the majority of patients were treated in the outpatient facility. Physical examinations, weight measurements, hemograms, and assessments of performance status were done on a weekly basis in the outpatient setting; other study parameters were repeated as often as necessary and at a minimum of every 6 weeks.

The eligibility requirements included anticipated survival of > 6 weeks, performance status of $\geq 30\%$, adequate marrow reserve in nonleukemic patients (wbc count $\geq 4000/mm^3$, platelet count $\geq 140,000/mm^3$, hemoglobin level ≥ 9 g/100 ml), and no serious impairment of hepatic, renal, or central nervous system function. Patients could not have received radiation therapy or chemotherapy within the 30 days preceding treatment with AMSA.

A patient was considered evaluable for toxic effects at a given dose level if he or she manifested toxic effects or completed a course of therapy (four weekly doses). Complete remission required complete disappearance of all evidence of disease with an improved (or normal) performance status for > 1 month. A partial remission was defined as a $> 50\%$ reduction in the sum of the products of the largest perpendicular diameters of all measurable disease accompanied by significant clinical improvement for ≥ 1 month. A minor response was defined as a $> 25\%$ but $< 50\%$ decrease in the sum of the products of measured tumor diameters.

Treatment was initiated at a dose of 20 mg/m^2, given iv weekly $\times$ 4 unless intervening toxic effects forced early discontinuance. Seventy-four courses were given to 34 patients, covering the dose range of 20–90 mg/m^2. Patients entered at dose levels of 20–70 mg/m^2 who tolerated 4 weeks of therapy without major toxicity were advanced to the next dose level.

The clinical preparation was a solution of AMSA in dimethylacetamide. This was diluted with 0.008 M lactic acid and was given directly or further diluted with 5% dextrose in water for infusion. The chloride salt of the drug precipitates from aqueous chloride-containing solutions. Initially, the drug was given as a 20-minute infusion in 50 ml of 5% dextrose in water. Due to local venous irritation with doses of ≥ 70 mg/m^2, the drug was diluted in 250 ml of 5% dextrose in water and infused over a 30-minute period, without subsequent evidence of phlebitis.

RESULTS

The dose-toxicity relationships observed are shown in table 3. Major side effects other than myelosuppression were not noted. Five of 34 patients experienced nausea and vomiting. On the day of injection, two patients developed malaise and myalgia lasting for 2 days.

Hematopoietic toxicity occurred inconsistently at 70 mg/m^2; it was dose-limiting at 90 mg/m^2. At 90 mg/m^2 the median leukocyte nadir was $2600/mm^3$ and occurred at a median of 17 days. The corresponding platelet nadir was $188,000/mm^3$ at 14 days. Recovery was rapid, occurring within 1 week. Only in rare instances was myelosuppression prolonged. Cumulative hematologic toxicity was not observed in patients cycled through more than one dose range.

In view of the fact that myelosuppression was observed in patients at all doses (20–90 mg/m^2), we reviewed the number of weekly doses received prior to evidence of toxic effects on the marrow (table 3). We were unable to explain the toxicity observed at low doses in terms of prior treatment (including previous therapy with nitrosoureas) or renal and hepatic function. At low doses, toxicity, when observed, occurred after three weekly doses. At 90 mg/m^2, ten of 12 patients had toxic reactions

TABLE 3.—Hematologic toxicity of AMSA

Weekly dose (mg/m^2)	No. of patients— Entered	Evaluable	With toxic effects*	Leukocyte nadir Cells × $10^3/mm^3$ Median	Range	Day	Platelet nadir Cells × $10^3/mm^3$ Median	Range	Day	Dose producing toxicity Median	Range
20	5	5	5	3.0	2.2–7.6	7	148	134–169	24	80	80
30	7	5	4	2.4	1.0–5.6	26	141	116–240	8	90	30–120
40	16	15	11	3.1	0.3–5.5	18	141	40–354	19	120	40–160
55	13	12	5	3.9,4.6	1.1–9.6	16,19	225,226	31–450	14	110	55–220
70	19	18	9	3.5,3.8	0.2–16.9	15,17	208,210	68–400	11,14	210	70–280
90	14	12	10	2.6,2.7	0.6–5.8	15,19	186,190	30–364	14	180	90–270

*Leukocyte nadir < $3500/mm^3$ or platelet nadir < $100,000/mm^3$.

and treatment was discontinued after only two weekly doses.

Twenty-two patients treated with AMSA were evaluable for antitumor effect. One patient with abdominal desmoid tumors experienced a partial remission which has been maintained for > 5 months. A patient with adenocarcinoma of the colon had a minor response consisting of shrinkage (< 50%) of supraclavicular nodes and a 50% decrease in carcinoembryonic antigen, lasting > 3 months to date.

DISCUSSION

This phase I trial of AMSA demonstrated dose-limiting toxic effects, which were solely hematologic, at 90 mg/m^2 given iv weekly × 4. Aside from hematopoietic toxicity the drug has been well-tolerated.

The findings in this phase I trial of AMSA in which the drug was given on a weekly basis are consistent with those reported by other centers where the drug was given on different schedules. Our findings suggest that a dose of 70–90 mg/m^2 would be suitable for trials of AMSA on a weekly basis.

The availability of a weekly schedule does not mandate its employment. To date, the clinical trials have not demonstrated acute dose-related toxic effects of AMSA that would limit the applicability of intermittent single-dose schedules in favor of a lower-dose weekly regimen. Although schedule-dependency for therapeutic effects has not been observed in AMSA-responsive murine tumor systems, it remains to be excluded in human neoplasms. A rigorous clinical evaluation of this question must await the demonstration of therapeutic activity in some human tumor type that could provide a focus for a comparison of dose schedules.

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Selective Inhibition of the Nephrotoxicity of *cis*-Dichlorodiammineplatinum(II) by WR-2721 Without Altering Its Antitumor Properties[1,2]

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SUMMARY

Injection of 200 mg/kg of body weight of WR-2721 30 minutes before graded doses of *cis*-dichlorodiammineplatinum(II) (cisplatin) increased the resistance of Fischer 344 rats to cisplatin-induced nephrotoxicity by a factor of 1.7, but did not alter the sensitivity of three different transplanted tumors to cisplatin-induced growth delay. As a consequence, it was possible to administer doses of cisplatin which would have been lethal in the absence of WR-2721 injection, resulting in a proportionally greater tumor response. WR-2721 can, therefore, increase the effectiveness of cisplatin therapy through selective protection against the toxic side effects of chemotherapy.

[Cancer Treat Rep 64:57–64, 1980]

In a recent study on the effects of WR-2721 injection on the toxic and antitumor effects of mechlorethamine (HN2) (1), it was demonstrated that injection of WR-2721 30 or 60 minutes before HN2 reduced host toxicity by factors of 2.0 and 1.9 respectively but did not alter the antitumor effectiveness of HN2. Only when the HN2 was given 5 or 15 minutes after WR-2721 was a reduced antitumor effect observed, and this was traced to inactivation of the HN2 in the circulation. The lack of protection of the tumor with the 30- and 60-minute spacings is presumably the result of deficient absorption of WR-2721 by the tumor (2,3) which also accounts for the ability of this drug to selectively protect normal tissues against radiation injury (4). The only difference between the potential application of WR-2721 in radiotherapy and chemotherapy is that with chemotherapy one must wait until serum levels of WR-2721 (and/or its metabolites) drop below those which can produce non-tumor site chemotherapeutic drug inactivation.

At the completion of these initial studies, we recognized that HN2 was neither the most effective nor the most general of chemotherapeutic agents, and that differences between it and other agents might limit the scope of this approach. We therefore initiated studies with a variety of drugs with the objective of determining whether WR-2721 pretreatment could protect the respective dose-limiting organs without altering the antitumor effectiveness of the respective drugs. We report here that WR-2721 can increase the resistance of rats to the nephrotoxic effects of *cis*-dichlorodiammineplatinum(II) (cisplatin) by a factor of 1.7, without altering the response of three solid tumors.

MATERIALS AND METHODS

Rats.—The rats used in the present study were Fischer 344 females aged 70 days at the time of treatment or tumor transplant. The rats were purchased commercially (Charles River Breeding Labs, Wilmington, MA) and housed five per cage, with food and chlorinated water provided ad libitum.

Tumors.—Three mammary tumors of the Fischer 344 rat were used in the present studies: the 3M2N squamous cell carcinoma, the R3230AC adenocarcinoma, and the 13762 adenocarcinoma (5). Approximately 10^6 tumor cells, harvested from sc tumors according to standard

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techniques (6), were transplanted sc into the right hind leg of the recipients. When the tumors reached an average diameter of ~ 10 mm, they were treated as described below. Following treatment tumor sizes were determined three times weekly until the tumors had grown 4 mm beyond their original size. Response is expressed as the drug-induced delay in days to reach this size.

Drugs.—WR-2721 [*S*-2-(3-aminopropylamino)-ethylphosphorothioic acid; sample AN] was kindly provided by Dr. Melvin S. Heiffer (Walter Reed Army Institute of Research, Washington, DC). The drug was dissolved in distilled water (20 mg/ml) and injected ip at a dose of 200 mg/kg. The dose of WR-2721 alone which kills 50% of the rats within 10 days is 476 ± 18 mg/kg. Rats given either saline or WR-2721 30 minutes earlier were given graded ip doses of cisplatin (Bristol Laboratories, Syracuse, NY). On Days 0, 3, 5, 7, and 10 following injection the BUN level, hematocrit value, and body weight were determined.

BUN assay.—Ward and Fauvie (7) reported that the pattern of rising BUN levels provided an accurate index of cisplatin-induced nephrotoxicity in male Fischer 344 rats aged 6 weeks. Following a dose of 7.5 mg/kg, the BUN levels rose to a maximum on Day 5 and then declined. Although our rats were from the same strain, they were females and 4 weeks older. We therefore first confirmed the observation of Ward and Fauvie (7) and then assayed the BUN patterns in our rats following cisplatin injection, with or without prior WR-2721 injection as above. At varying intervals after treatment, each rat was bled (150 µl) from the supraorbital plexus, with the blood being harvested in heparinized capillary tubes (Dade, Miami, FL). Following centrifugation, the hematocrit value was determined and 10 µl of plasma was harvested for analysis. All reagents for the BUN analysis were purchased from Sigma Biochemicals (St. Louis, MO). The plasma was added to 0.16 unit of urease in 0.5 ml of buffer (catalog No. 640-5) and incubated for 15 minutes in a 37°C water bath. To the sample was then added 1 ml of phenol nitroprusside reagent (catalog No. 640-1), 1 ml of alkaline hypochlorite (catalog No. 640-3), and 5 ml of double-distilled water. After allowing 30 minutes for color development, the absorbance of the samples was determined at 570 nm. The relationship between BUN concentration and absorbance was linear over the 0–60-mg/100 ml range. Samples beyond the linear range were diluted appropriately and re-analyzed.

RESULTS

Figure 1 is a plot of the observed 30-day mortality as a function of cisplatin dose for rats given saline or 200 mg/kg of WR-2721 30 minutes before cisplatin. Although nephrotoxic deaths normally occur between Days 5 and 10 following injection in control animals (7), we expressed the data in terms of 30-day mortality to point out that WR-2721 had not merely postponed death or that another syndrome had not appeared. All deaths occurred between the third and seventh days after injection, with each being preceded by typically poor gross appearance (7). These data are too limited to warrant precise calculation of toxic LD50 values, but it is apparent from the data (fig 1) that WR-2721 has protected the rats against cisplatin-induced lethality.

Figure 2 contains the body weight change on Days 3 (fig 2A) and 7 (fig 2B) as a function of cisplatin dose for control and WR-2721-pretreated rats. No protection by WR-2721 is apparent on Day 3 (fig 2A) but by Day 7 the WR-2721-pretreated rats show greater body weight recovery at both the 5-mg/kg ($P < 0.20$) and 7.5-mg/kg ($P < 0.02$) doses. This loss of body weight by Day 3 would appear to be due to dehydration. In support of this suggestion, the Day-3 hematocrit value is a positive linear function ($r = 0.86$) of the cisplatin dose (fig 3), and again, no protection by WR-2721 is apparent.

We assayed the BUN levels in both groups of rats as a function of cisplatin dose and time after injection. BUN levels rose by Day 3 (fig 4), peaked on Day 5 (fig 5), and declined thereafter, independent of cisplatin dose or WR-2721 pretreatment. No data are available after Day 3 for saline-treated rats given 10 mg/kg of cisplatin, since all had died by the morning of the fifth day. It requires approximately 3.5 mg/kg more of cisplatin to induce any particular BUN level on either day in WR-2721-pretreated rats than in controls. This translates into a dose reduction factor of 1.7, relative to a 5-mg/kg dose given to saline-pretreated rats.

Given significant protection of the kidney, the question becomes whether WR-2721 pretreatment will alter the antitumor effectiveness of cisplatin. Our present understanding of how WR-2721 achieves selective protection (1,4) and our experience with HN2 (1) and with cyclophosphamide (Yuhas JM, unpublished data) all suggest that WR-2721 would not protect tumors against cisplatin, yet we wished to verify this experimentally.

To test this we determined the growth delay in three Fischer 344 rat tumor systems following a 5-mg/kg cisplatin dose in control animals and a 5- or 7.5-mg/kg cisplatin dose in WR-2721-pretreated rats. Note that control animals would not tolerate the 7.5-mg/kg cisplatin dose (fig 1). The results of this experiment are summarized in table 1 and demonstrate quite clearly that WR-2721 does not protect any of the tumors against the 5-mg/kg dose of cisplatin and that the increased tolerance to cisplatin in WR-2721-pretreated rats allows the administration of 7.5 mg/kg of cisplatin and provides a proportional increase in the response of the tumor.

DISCUSSION

The data presented above indicate that pretreatment with 200 mg/kg of WR-2721 30 minutes before cisplatin

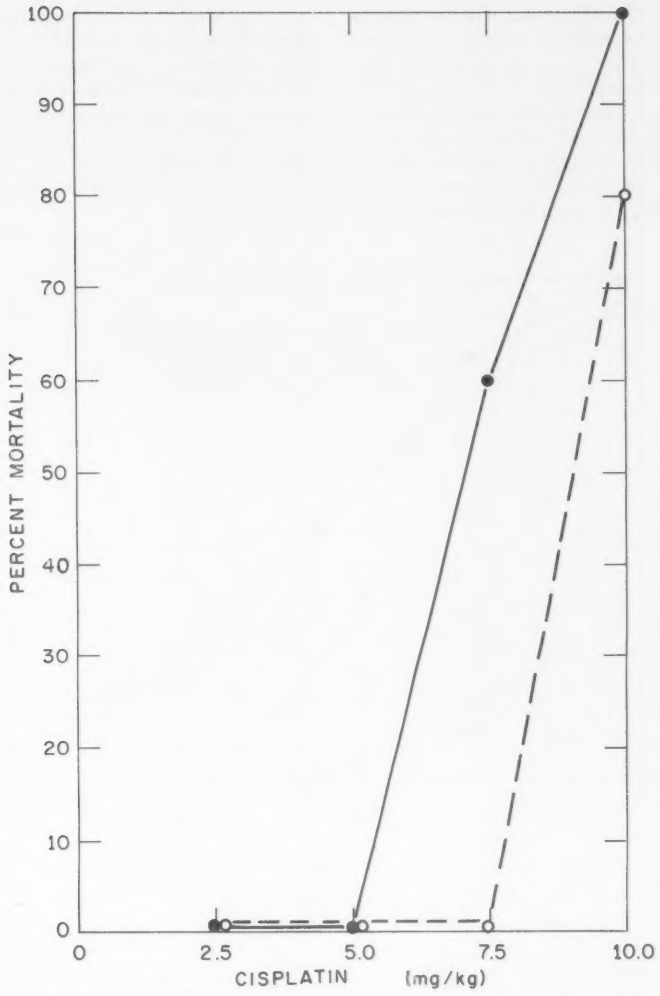

FIGURE 1.—% mortality at 30 days after injection of graded doses of cisplatin in Fischer 344 rats given saline (●) or 200 mg/kg of WR-2721 (○) 30 mins before cisplatin.

injection can increase the resistance of rats to nephrotoxicity by a factor of 1.7, without altering the response of their tumors. Data presently accumulating in our laboratory indicate that similar, although reduced protection can be obtained with WR-2721 doses as low as 50 mg/kg, and that increasing the WR-2721 dose to > 200 mg/kg increases the protection against nephrotoxicity. Further, WR-2721 is being tested using cisplatin treatment protocols of weekly treatment until death or five daily treatments. In these studies no tumor protection has been observed, and the dose reduction factors for protection against nephrotoxicity are all larger than the 1.7 value observed in the single-dose studies above. These studies are presently being expanded and will be reported elsewhere.

These studies indicate that WR-2721 pretreatment could increase the effectiveness of chemotherapy either by allowing the cisplatin dose per treatment to be increased or by allowing standard dosing to be continued for a longer period. Which of these approaches would be most advantageous would depend on the characteristics of the tumor being treated. If present protocols are fighting a continually losing battle against a particular tumor

type, then increasing the individual dose of cisplatin would be required. Alternatively, if present protocols appear to be controlling the tumor adequately but host toxicity forces discontinuation of treatment, the alternate approach would appear ideal. From a strictly practical standpoint, this latter approach would be simplest for safe clinical testing. Patients could be evaluated after the standard treatment course and if WR-2721 had protected the kidneys the treatments could be continued. Alternatively, if protection was not observed, treatment could be discontinued with no additional risk to the patient over that encountered in a standard treatment course. Based on our studies of the mechanisms underlying selective normal tissue protection against chemotherapy, however, which have involved mice, rats, and rabbits, we consider it highly unlikely that similar selectivity would not be observed in patients (Yuhas JM, unpublished data).

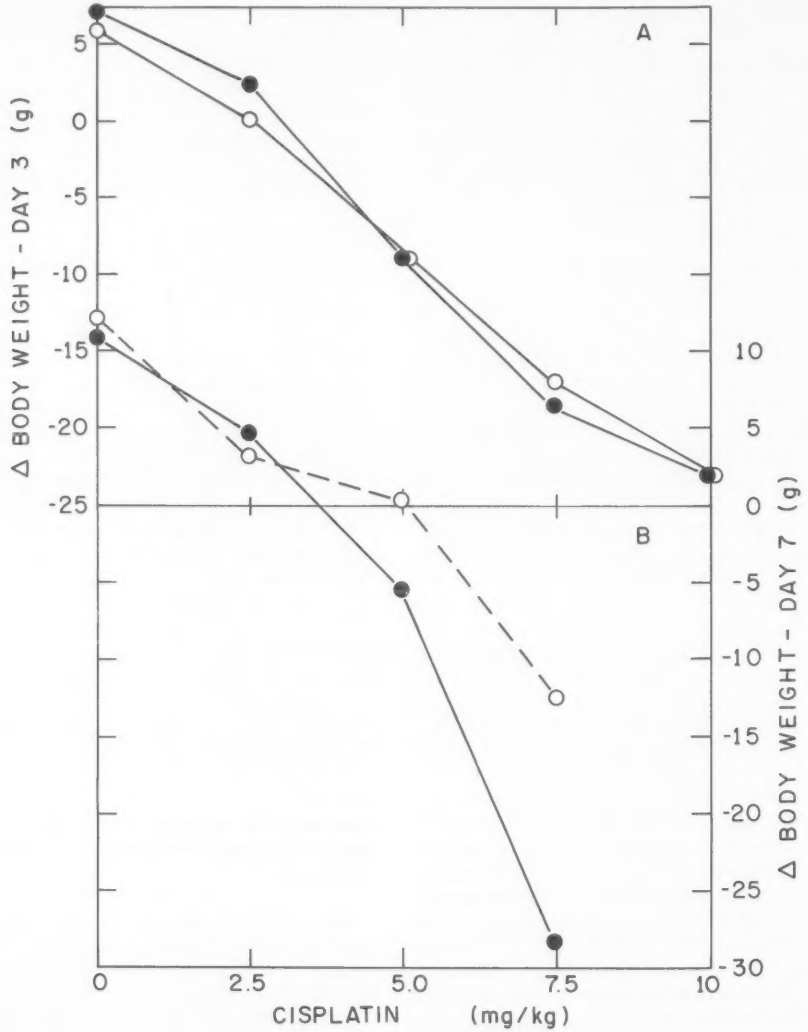

FIGURE 2.—Change in body weight on Days 3 (A) and 7 (B) following injection of cisplatin in rats pretreated as in fig 1 with saline (●) or WR-2721 (○).

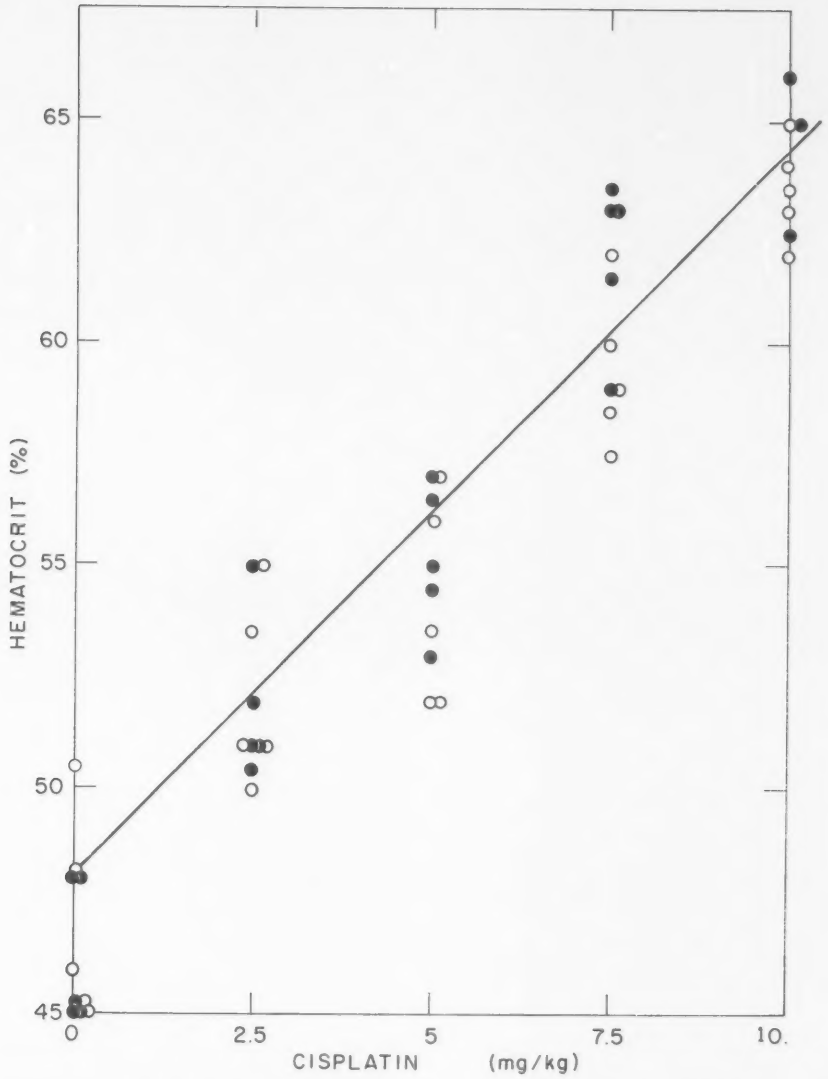

FIGURE 3.—Hematocrit values for individual rats on Day 3 after graded doses of cisplatin. ● = saline-pretreated animals; ○ = WR-2721-pretreated animals.

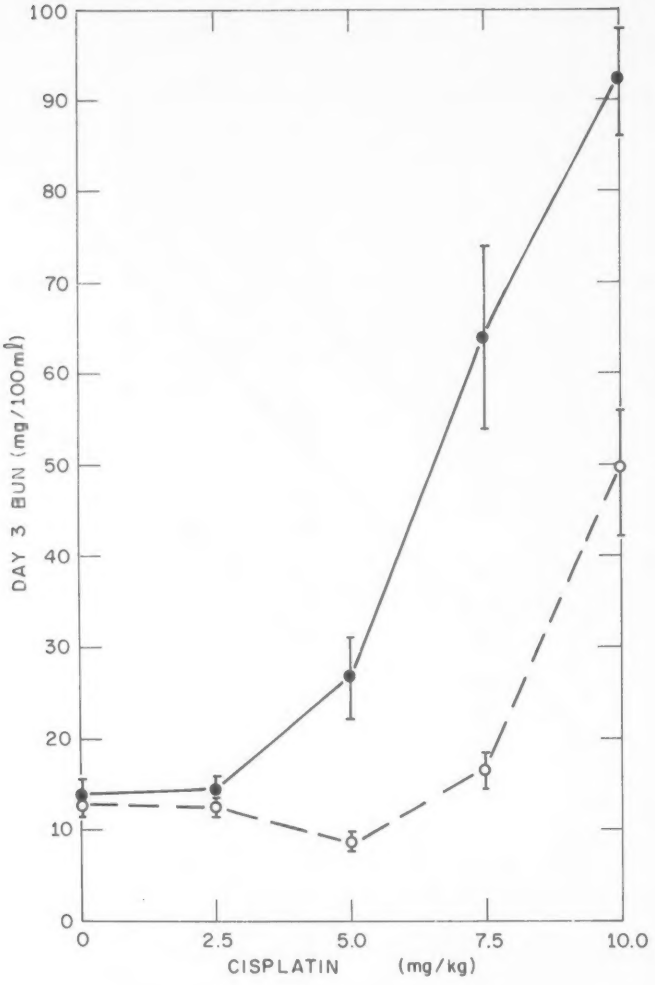

FIGURE 4.—BUN levels on Day 3 as a function of cisplatin dose for rats pretreated with saline (●) or WR-2721 (○).

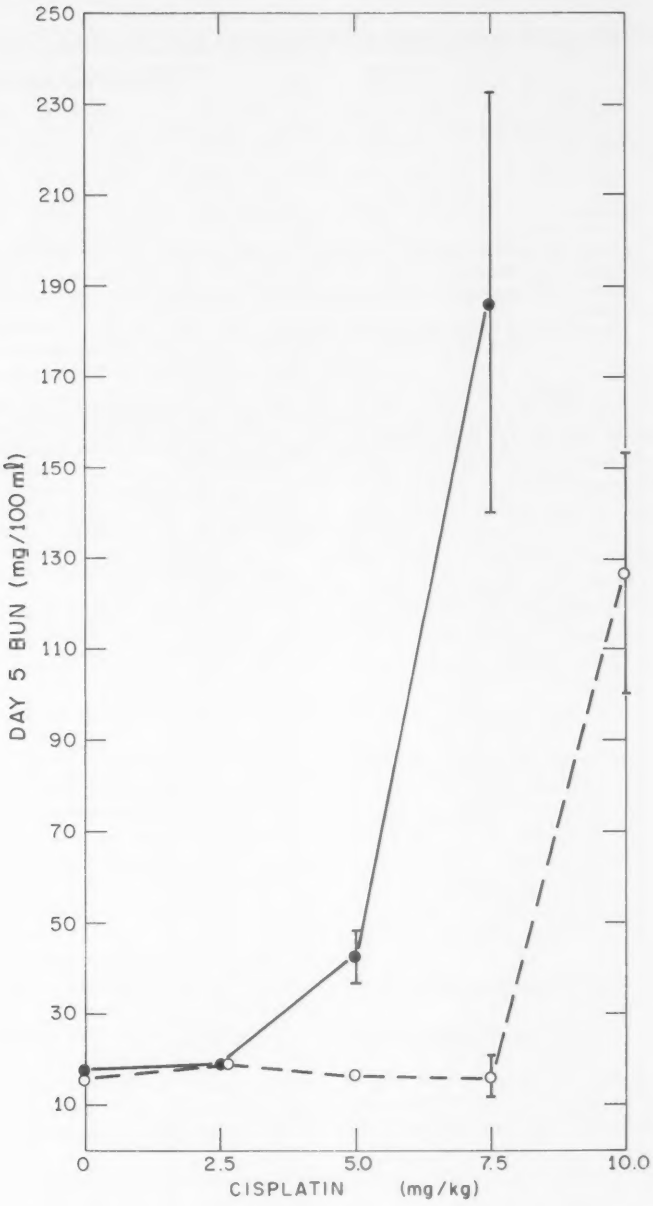

FIGURE 5.—BUN levels on Day 5 as a function of cisplatin dose for rats pretreated with saline (●) or WR-2721 (○).

TABLE 1.—Cisplatin-induced delay in the time required for 10-mm tumors to grow an additional 4 mm

Tumors*	Delay in growth (days ± SE) for—		
	Control rats receiving 5 mg/kg of cisplatin	WR-2721-pretreated† rats receiving cisplatin at—	
		5 mg/kg	7.5 mg/kg
3M2N	6.0 ± 0.52	6.1 ± 0.81	10.0 ± 1.10
13762	8.3 ± 0.37	7.9 ± 0.66	12.6 ± 0.91
R3230AC	2.4 ± 0.36	3.2 ± 0.71	5.0 ± 0.18

*WR-2721 pretreatment alone did not induce tumor growth delay; pooled data for saline- and WR-2721-pretreated rats were as follows for the time required to grow from 10 to 14 mm: 3M2N = 3.1 days; 13762 = 4.3 days; and R3230AC = 8.4 days.

†200 mg/kg by ip injection 30 mins before cisplatin.

Since iv hydration is used clinically to reduce the cisplatin concentration in the kidney, and presumably the tumor, we are currently studying the interaction of this approach with WR-2721 pretreatment in order to determine whether they could be used in conjunction.

As a last point, it must be demonstrated that patients can tolerate protective doses of WR-2721. These data should be forthcoming in the near future since phase I studies on WR-2721 are presently underway in both Japan and New Mexico.

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Combination of Levamisole Immunotherapy and Polychemotherapy in Advanced Breast Cancer[1]

P. Klefström[2,*]

SUMMARY

In a randomized double-blind study, 101 female patients with breast cancer treated with Adriamycin chemotherapy in combination with vincristine and cyclophosphamide received either levamisole immunotherapy or placebo. The investigation was preceded by a pilot study involving 44 patients, of whom 25 received levamisole; the intermittent four-drug schedule of cyclophosphamide, vinblastine, methotrexate, and 5-fluorouracil, however, formed the basis of chemotherapy. In both studies, levamisole increased the response rates ($P = 0.03$), and in the present controlled trial it also significantly lengthened survival ($P = 0.05$). The results correlated with initial and levamisole-induced improvement of cell-mediated immunity.

[Cancer Treat Rep 64:65–72, 1980]

The development of combination chemotherapy programs for advanced breast cancer has produced encouraging increases in remission rates (1–3). Remission durations have, however, remained short, with a median of 5–10 months. Although the anticancer chemotherapies have proved to be immunosuppressive (4,5), the immunocompetence of patients has also correlated with the treatment responses (6–9). Sadoff has proposed an alternative hypothesis regarding the mechanism of cytotoxic drugs, based on inhibition of immunologic function (10). Appropriate doses of drugs may inhibit the blocking factors but not the function of cytotoxic T cells. Therefore, immunopotentiating treatments in addition to chemotherapy are of topical interest. Gutterman et al (11), for example, used bacillus Calmette-Guérin with 5-fluorouracil, Adriamycin, and cyclophosphamide (FAC), obtaining significantly better remission duration and survival rates compared to earlier results with FAC alone.

At the Radiotherapy Clinic of Helsinki University Central Hospital, a four-drug combination (modified from Hanham et al [2]) with cyclophosphamide, vinblastine, methotrexate, and 5-fluorouracil (CVMF) was used in breast cancer. This chemotherapy schedule was combined with levamisole in a pilot study initiated in October 1975. Levamisole proved to be a safe synthetic compound (12), and was easier to use than vaccines because of the possibility of oral administration. It was found to be an antianergic drug in cases where immunocompetence was diminished by age, drugs, or disease.[3] Thus, with the aim of reversing the immunosuppression caused by chemotherapeutic agents, it was chosen for the study. Since the results of the pilot study were encouraging, a randomized double-blind investigation using levamisole and placebo was conducted. Adriamycin combination therapy was chosen for this controlled study because of its well-documented efficacy in breast cancer (3,13,14).

PATIENTS AND METHODS

Patient Selection

One hundred and one female patients with disseminated breast cancer were treated with Adriamycin combination chemotherapy. The study was randomized and double-blind with respect to immunotherapy with levamisole. Forty-eight patients received levamisole plus

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TABLE 1.—Schedules of treatment regimens

Regimen	Drug	Dose	Day of treatment 1	2	3	4	5	6	
CVMF	5-Fluorouracil	500 mg*	×	×	×	×	×		Repeat q4wks
	Methotrexate	12.5 mg*	×			×			
	Cyclophosphamide	300 mg*	×				×		
	Vinblastine	6 mg*		×			×		
VAC	Adriamycin	40 mg/m²	×						Repeat q4wks
	Vincristine	1 mg	×						
	Cyclophosphamide	150 mg/m² orally		×	×	×	×	×	
	+								
	Levamisole, 2.5 mg/kg/day for 2 consecutive days, given each wk except wk when chemotherapy is given								

*These doses are for a 60-kg patient.

chemotherapy, and 53 patients received placebo plus chemotherapy. The trial was preceded by a pilot study of 44 patients, of whom 25 received levamisole plus CVMF chemotherapy.

Female patients with metastases of or recurrent breast cancer were admitted to the study if the following conditions were met: < 75 years old, no prior nonhormonal chemotherapy or immunotherapy, no other malignancies in their medical histories, no mental illness, disease which was not so advanced that the patients were unable to tolerate treatment and thus died before the third course of chemotherapy (2 months), and able to care for themselves (Karnofsky score of at least 60%). Three patients in the pilot study and four in the controlled study were excluded because of early death or refusal to receive treatment. Randomization was arranged in the pilot (CVMF) study so that patients with birthdays on even dates received levamisole. In the controlled (vincristine, Adriamycin, and cyclophosphamide [VAC]) trial, the patients received a coded drug package which included either levamisole or placebo.

Treatment Schedule

In the pilot study, intermittment cyclic polychemotherapy (CVMF) was used (table 1). The patients received this treatment 1 week out of 4, provided that the leukocyte and platelet counts had recovered during the intervening 3 weeks. In the controlled trial, Adriamycin and vincristine were chosen first for the chemotherapy schedule. Patients received vincristine (1 mg) on the first day and Adriamycin (60 mg/m²) divided between the 2 following days. In October 1976, cyclophosphamide was added to the treatment schedule (13,14). It was given orally (150 mg/m²/day) on Days 2–6, and the dose of Adriamycin was reduced to 40 mg/m² on Day 1 (table 1). Adriamycin treatment was discontinued if any signs of cardiotoxicity appeared. The total dose of Adriamycin was limited to 500 mg/m² because of the risk of cardiotoxicity. In both studies the patients received levamisole or placebo on Day 8 after the start of chemotherapy. The dose of levamisole was 2.5 mg/kg/day on 2 consecutive days a week, given every week except those when chemotherapy was given. The initial treatment plan was to give six courses of chemotherapy if remission was achieved, and to continue levamisole for a total of 1 year. However, towards the end of the study it became clear that it would be necessary to give at least three more courses, even where complete remission (CR) had been achieved. However, because of the randomization method in the controlled trial no differences in chemotherapy resulted between the immunotherapy and control groups. If no response was achieved after three courses of chemotherapy, the treatment then became individualized. Usually CVMF was used as nonhormonal chemotherapy in patients not responding to Adriamycin and vice versa.

Immunocompetence Tests

The immunocompetence of the patients was measured by skin tests using purified protein derivative (PPD) as an antigen injected intradermally in concentrations of 0.1 and 1.0 tuberculin unit. Positivity to PPD was expected (at least to some PPD concentrations) in most of the patients because tuberculosis has been a common disease in Finland and most Finnish people have been vaccinated with bacillus Calmette-Guérin. Another test used was cytologic blast cell transformation with phytohemagglutinin.

The tests for immunocompetence were made just before the first course of chemotherapy. If remission or stabilization was achieved, the tests were repeated 1 month after the sixth course of chemotherapy. If the tumor appeared to grow, or if any new metastatic lesions appeared and the disease did not respond to the treatment, the tests were repeated earlier than planned, although at least 1 month after the last treatment with cytostatic agents.

TABLE 2.—Clinical features of patients in the different treatment groups

	VAC with levamisole	VAC control	CVMF with levamisole	CVMF control
Total No. of patients	48	53	25	19
Median age (range) in yrs	56(19–69)	53(37–74)	54(38–70)	63(39–74)
Median disease-free interval (range) in mos	23(0–112)	16(0–109)	21(0–75)	26(1–120)
No. of premenopausal patients at diagnosis	18(38%)	19(39%)	11(44%)	4(21%)
No. of patients with previous hormone treatments	19(40%)	23(43%)	10(40%)	8(42%)
Site of metastases (No. of patients)				
Lymph nodes	6	2	5	4
Skin	4	6	6	5
Bones	18	20	3	3
Lung + pleural	16	16	6	8
Liver	7	10	7	5
NED*	8	8	3	1

*NED = no measurable lesions at the start of treatment.

Response Definitions

CR was defined as the disappearance of all manifestations of disease; partial remission (PR) was defined as a $\geq 50\%$ reduction in measurable tumor size and partial recalcification of bone lesions with simultaneous pain relief and decrease of pathologic changes in bone or liver scan; stabilization was defined as a $< 50\%$ reduction in tumor size or subjective pain relief in bone metastases without obvious progression as shown on bone roentgenogram or bone scan or an increase in the serum alkaline phosphatase level; progression was defined as any local increase in tumor size or number of metastases even in cases of simultaneous regression in other areas. This study included some patients who had no measurable tumors at the beginning of the treatment. These patients' metastases or recurrences had been treated by operation or radiotherapy, but, because of the high risk of dissemination, "adjuvant" treatment with cytostatic agents was deemed necessary. Duration of remission was determined from the start of the treatment to the first appearance or progression of any tumors or metastases in patients with CR or PR, as well as in the patients with no measurable tumors. The survival time in the life tables was also determined from the beginning of the treatment.

Clinical Features of the Study Groups

The clinical features are comparable in both groups receiving Adriamycin chemotherapy with or without immunotherapy. There was no statistically significant difference between the groups with respect to any of the clinical features listed in table 2 (P values ranged from 0.3 to 0.86). In the pilot study all clinical features were similar in the levamisole and control groups except for median age, which was significantly higher in the control group.

Statistical Methods

Survival curves were constructed according to the life-table method using the computer program described by Peto et al (15,16). The program also evaluated the differences in survival between the groups using the log-rank or Mantel-Haentzel test. When two groups are compared, the second chi-square value, for "trend," includes a correction for continuity. The third chi-square value is the test for departures from the trend. When calculating the usual chi-square values from the tables, Yate's correction was included.

RESULTS

Results of the Pilot Study

The small number and heterogeneity of the patients in the pilot (CVMF) study do not permit the determination of significant differences in the duration of remission and in life-expectancy tables (figs 1 and 2). There was, however, a trend in favor of the levamisole treatment. The remission rate (table 3) was significantly better ($P < 0.05$) in patients receiving levamisole than in the control or historical control patients.

Results of the Controlled (VAC) Trial

The response rates were significantly better ($P = 0.03$) in the VAC plus levamisole group than in the VAC plus

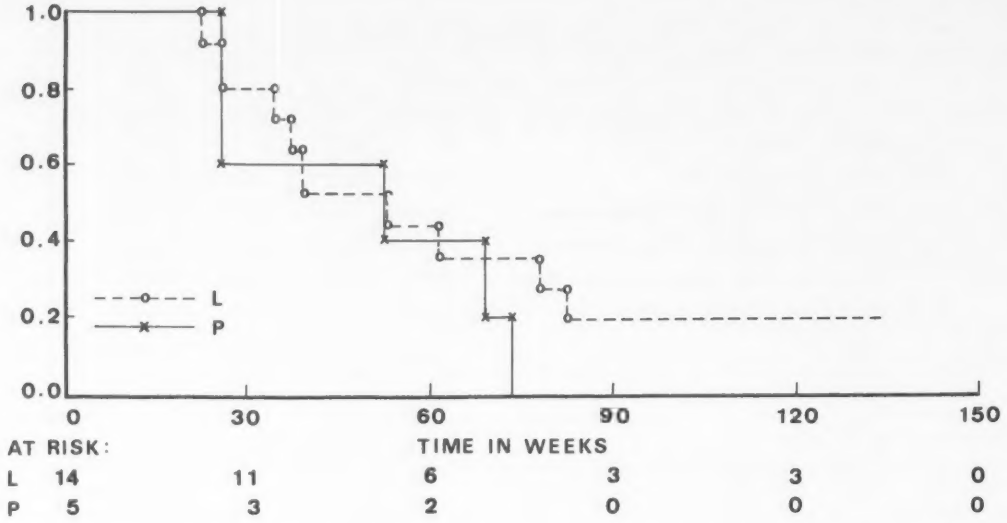

FIGURE 1.—Duration of remission in patients receiving CVMF-levamisole (L) and CVMF alone (P). The difference is not significant.

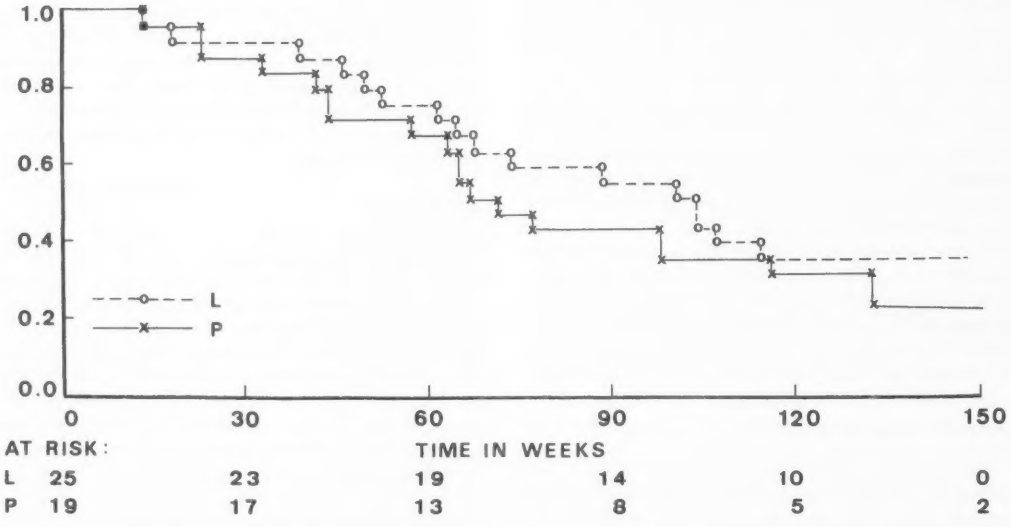

FIGURE 2.—Survival of patients recieving CVMF-levamisole (L) and CVMF alone (P). The difference is not significant.

TABLE 3.—Overall response rates to treatments

Response	No. of patients (%) treated with—			
	VAC with levamisole	VAC control	CVMF with levamisole	CVMF control
Remission	25(63)	21(47)	11(50)	4(22)
Complete	11(28)	5(11)	4(18)	1(5)
Partial	14(35)	16(36)	7(32)	3(17)
Stabilization	10(25)	9(20)	6(27)	6(33)
Progression	5(12)	15(33)	5(23)	8(45)

TABLE 4.—Overall response according to site of metastases

Treatment	No. of patients with response/No. with metastases in—				
	Lymph nodes	Skin	Bones	Lungs	Liver
VAC with levamisole	4/6	2/4	10/18	14/16	5/7
VAC control	0/2	1/6	10/20	10/16	6/10
CVMF with levamisole	3/5	3/6	2/3	2/6	4/7
CVMF control	1/4	0/5	0/4	3/7	2/5

placebo group (table 3) when remissions were compared to disease progressions. Of those eight patients in the immunotherapy group having no measurable metastases before treatment, three developed recurrences in earlier treated areas. One patient who developed distant metastases had to stop levamisole treatment because of granulocytopenia. Three of the eight control (placebo) patients with no measurable metastases before treatment developed distant metastases. Two of them died. Table 4 shows the response at the different sites of metastases. The treatment response of patients with lymph node, liver, and lung metastases was markedly improved by levamisole. In the chemoimmunotherapy group 14 of 16 patients with lung metastases responded to the treatment by achieving remission, and in the remaining two the disease stabilized. The remission rate of patients with bone metastases was not high (approximately 50%) in either the chemotherapy or chemoimmunotherapy group. Patients with metastases in the skin and subcutaneous tissue also responded poorly, but most patients in this group were treated earlier with postoperative radiotherapy.

The difference in favor of levamisole with respect to duration of remission is not significant (fig 3). Only those patients who responded at least partially to the treatment and the patients without measurable lesions at the start of the treatment are compared. One control patient to whom levamisole was given by mistake after six courses of chemotherapy was excluded.

The difference in survival (fig 4) in favor of levamisole is significant ($P = 0.05$). All evaluable patients are compared.

Response With Respect to Hormone Receptors

Hormone receptors were determined in seven patients in the VAC treatment groups. Positive estrogen and progesterone receptors were found in five patients and none of them responded to treatment. Four of these five patients were in the levamisole group.

Results With Respect to Immunocompetence

In both studies there was a positive correlation between immunocompetence and remissions achieved. Figure 5 reveals significant differences in survival between patients with positive and negative skin tests. However, the survival of those patients who were anergic before treatment, but received immunopotentiating therapy with levamisole, was the same as the survival of patients with positive reactions. In the VAC-levamisole group, potentiation of PPD skin tests occurred in all patients with CR, apart from the two who were already positive to 0.1 tuberculin unit before treatment. Potentiation of skin tests did not occur in the control VAC group or in the patients who received VAC-levamisole and did not respond. The same trend could be seen in the phytohemagglutinin stimulation tests, but because of the inaccu-

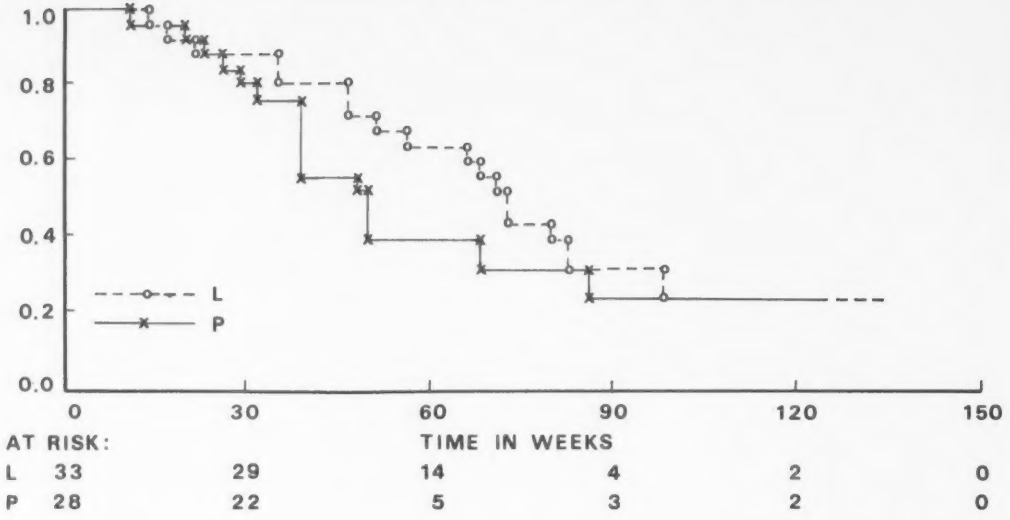

FIGURE 3.—Duration of remission in patients receiving VAC-levamisole (L) and VAC-placebo (P). The difference is not significant.

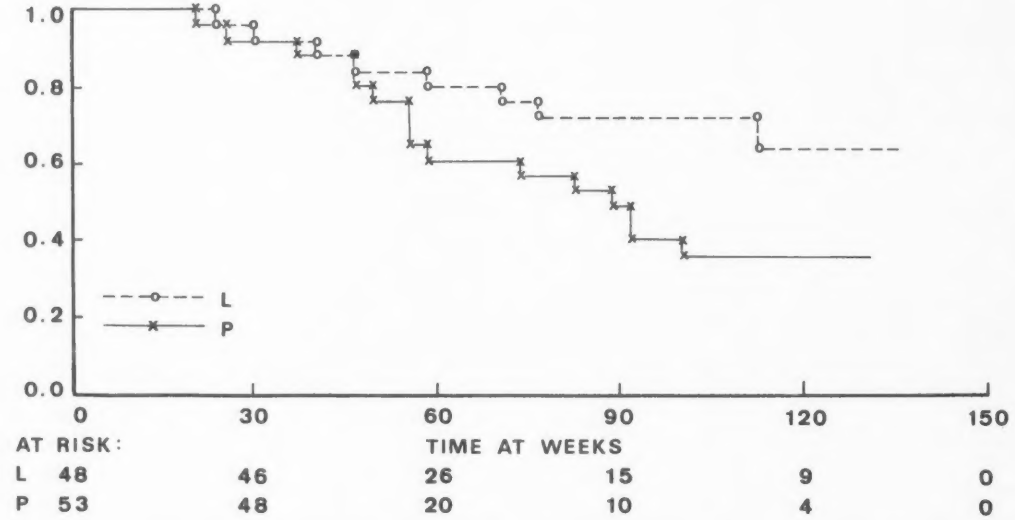

FIGURE 4.—Survival of patients receiving VAC-levamisole (L) and VAC-placebo (P). The difference in favor of VAC-levamisole is significant ($P = 0.05$).

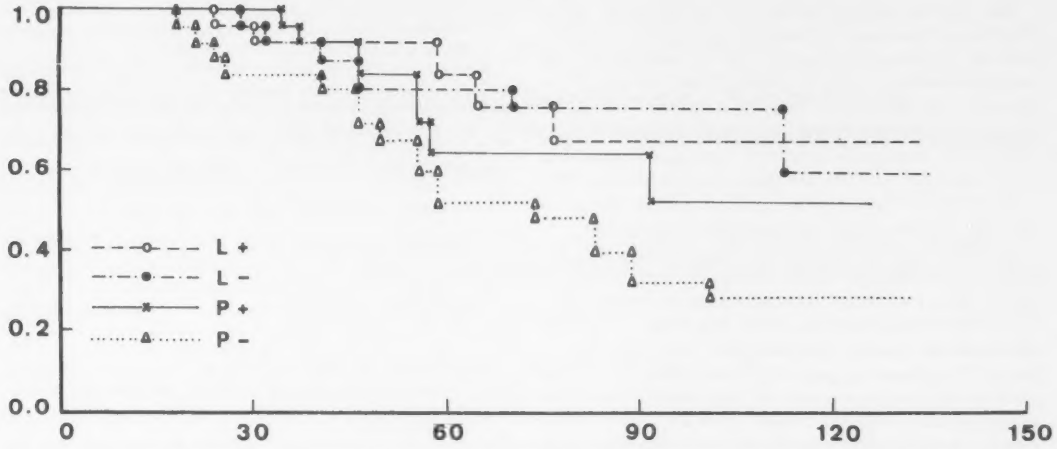

FIGURE 5.—Survival of patients with respect to immunocompetence before treatment measured by PPD skin tests. L+ = patients receiving levamisole with positive skin tests; L– = patients receiving levamisole with negative skin tests; P+ = patients receiving placebo with positive skin tests; and P– = patients receiving placebo with negative skin tests. The difference in favor of positive skin tests and immunopotentiating therapy is significant ($P = 0.016$).

racy of the morphologic method (most tests yielded a normal response), there were no statistical differences.

Side Effects

The chemotherapy regimens proved to be quite well-tolerated. Nausea, vomiting, and bone marrow depression occurred, but it was not usually necessary to stop the treatment or lower the doses. Leukocyte and thrombocyte counts usually recovered to normal within 3 weeks. Alopecia occurred in almost all patients in the Adriamycin treatment group. One patient had to stop the Adriamycin treatment because of its cardiotoxicity.

Most patients experienced no side effects from levamisole. However, eight patients developed agranulocytosis. Five of them were in the VAC group. The diagnostic criterion for agranulocytosis was that the leukocyte count did not recover after chemotherapy before levamisole treatment was interrupted. Agranulocytosis without any depression of other blood cells was then found both in peripheral blood and bone marrow. After cessation of levamisole treatment, bone marrow recovered during the following 2–3 weeks without fatal complications. The age range of the patients with agranulocytosis was 19–70 years. Seven patients were > 50 years old.

Agranulocytosis appeared between 2 and 22 months after the beginning of treatment. All patients but one were anergic in PPD skin tests. There were no correlations with HLA-type or other treatment or disease.

In eight patients in the VAC group, levamisole treatment was stopped because of other side effects. Five had granulocytopenia. Two patients developed allergic skin reactions and one developed nausea and gastric pain. One patient with CR in the VAC-levamisole group developed cerebral atrophy.

DISCUSSION

The rationale for tumor immunotherapy is based on the suggestion that it is possible to support "immunologic surveillance" and also to correct the detrimental balance between tumor and host. In addition to other treatments, levamisole is one possibility for supportive immunotherapy. In a review of experimental and clinical data on levamisole, Amery et al (12) made the following points: levamisole is an antianergic drug, which preferentially affects metastatic formation; tumor enhancement is unlikely with levamisole; and in animal models levamisole has been shown to improve the treatment results of chemotherapy, but only when added after chemotherapy. The results of this study seem to correlate quite well with those observations. The response rates were better in both studies with levamisole-treated patients. The overall survival in the controlled VAC study was significantly ($P = 0.05$) prolonged by levamisole. The best effect on survival occurred in patients receiving levamisole who responded to treatment. The median survival time increased from 98+ to 135+ weeks (approximately 50%) in patients with CR, from 92 to 130+ weeks (> 41.3%) in patients with PR, from 88 to 112 weeks (27.3%) in patients with stable disease, and from 57 to 71 weeks (24.6%) in patients with disease progression. The response rate and survival also correlated with initial and levamisole-induced immunocompetence.

One possible reason for the lack of difference in the duration of remission is that the treatment schedules, both for chemoimmunotherapy and the subsequent levamisole treatment, were too short. These studies include patients who, after good primary response to immunopotentiation, relapsed with decreased skin reactivity to PPD when levamisole treatment was discontinued, but who responded again to the chemoimmunotherapy with recovery of skin test reactivity. However, the remission duration has been evaluated to the first recurrence.

Recently, two independent investigators (17,18)[1] using levamisole in addition to polychemotherapy in advanced breast cancer reported the same kind of results. In a prospective randomized study by Stephens,[1] levamisole increased the overall remission rate, survival, and duration of remission in patients treated with FAC. The same observations were made by Hortobagyi et al (17,18) except in the remission rates. Hortobagyi used higher doses of drugs and the response rate in the chemotherapy group was better than in this study. Levamisole seems to raise the remission rate to the same level (about 70%) even when submaximal doses of cytostatic drugs have been used.

The high risk of agranulocytosis (19) demands careful monitoring of wbc counts when levamisole is used. However, those patients who developed agranulocytosis survived exceptionally long when compared to other patients and especially when compared to those who did not respond better than the agranulocytosis patients. A group in Miami found the same correlation in a similar study (Amery WK, personal communication, 1979). Therefore, in spite of the risk of agranulocytosis the results are encouraging enough for levamisole to be proposed as a good alternative to other immunotherapy models.

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Comparison of Two Combination Chemotherapy Regimens for Multiple Myeloma: Methyl-CCNU, Cyclophosphamide, and Prednisone Versus Melphalan and Prednisone[1]

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SUMMARY

Of 139 evaluable and previously untreated patients with multiple myeloma, 67 received methyl-CCNU-cyclophosphamide-prednisone (group A) and 72 received melphalan-prednisone (group B); 48% and 33% respectively had good responses and the overall response rates (good plus partial) were 75% and 65% for groups A and B respectively. The survival curves for both groups of patients were similar, with a median survival of 32 months. At 36 months, 70% of those patients who obtained good response were alive, 29% of those with partial response were alive, and 13% of those with no response were alive. The clinical staging system described by Durie and Salmon shows a good prognosis for stage I patients, with 80% remaining alive at 48 months, while the survival curves for stage II and III patients were similar, with 33% and 28% respectively remaining alive at 48 months. The combination of methyl-CCNU-cyclophosphamide-prednisone is not more effective in terms of response rate or duration of survival than melphalan-prednisone.

[Cancer Treat Rep 64:73–79, 1980]

Melphalan is an alkylating agent used since 1958 for the treatment of multiple myeloma. Alexanian et al (1) reported a greater response in patients treated with melphalan-prednisone than in patients treated with melphalan alone. Also, patients treated with the combination of both drugs have a longer survival. Cyclophosphamide has been reported to have therapeutic activity in multiple myeloma similar to that of melphalan (2). More recently, the nitrosoureas have been shown to be effective in the treatment of this disease (3). Several combinations of two, three, and four drugs are being used in randomized trials by cooperative groups (4,5).

At present there is no clear evidence that, as in lymphoma and acute leukemia, the combination of several drugs increases the response and survival (6) of patients with multiple myeloma. In October 1973 a controlled ther-

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[2]This study was conducted by the Grupo Argentino de Tratamiento de la Leucemia Aguda. The following institutions and investigators participated in the study: Instituto de Investigaciones Hematológicas "Mariano R. Castex," Academia Nacional de Medicina, Buenos Aires, Argentina (*A. Pavlovsky, S. Pavlovsky, G. Garay,* and *M. Tezanos Pinto*); Instituto Municipal de Hematología, Hospital Ramos Mejía, Buenos Aires, Argentina (*G. Bomchil, J. Turenski, J. Weingast,* and *J. Becherini*); Centro de Educación Médica e Investigación Clínica, Buenos Aires, Argentina (*A. Suárez* and *R. Cacchione*); Hospital Escuela "Gral. San Martín," Buenos Aires, Argentina (*C. A. Barros, J. Pileggi, H. de María, C. Russo, M. Curutchet,* and *M. A. Pizzolato*); Policlínico "Mariano Castex," Buenos Aires, Argentina (*M. Palau* and *S. C. de Sica*); Instituto "Gral. San Martín," Buenos Aires, Argentina (*L. Bergna*); Instituto de Oncología "Angel H. Roffo," Buenos Aires, Argentina (*A. Audino Pavlovsky*); Instituto Modelo de Clínica Médica "Luis Agote," Hospital Rawson, Buenos Aires, Argentina (*A. Macchi* and *C. Jait*); Hospital "Teodoro Alvarez," Buenos Aires, Argentina (*F. J. Cavagnaro, A. B. S. de Huberman, R. Urribarri,* and *R. Bezares*); Hospital "Clemente Alvarez," Rosario, Argentina (*J. Saslavsky* and *J. M. Lein*); Hospital Ferroviario Central, Buenos Aires, Argentina (*E. Quiroga Micheo, S. I. Calabria,* and *S. de Rudoy*); Hospital Militar Central, Buenos Aires, Argentina (*A. Musso* and *M. I. Santos*); and Instituto de Investigaciones Médicas, Hospital Tornú, Buenos Aires, Argentina (*J. C. Sanchez Avalos, A. Díaz,* and *F. Molina*).

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apeutic trial using two regimens (methyl-CCNU [MeCCNU]-cyclophosphamide-prednisone and melphalan-prednisone) was started, with the purpose of evaluating the response rate and survival of these two combinations.

MATERIAL AND METHODS

Between October 1973 and November 1976, 139 previously untreated patients with multiple myeloma were treated by eight member institutions of the Argentine Group for the Treatment of Acute Leukemia. Followup of the patients entered in this study has been conducted at regular intervals and the analysis is based upon data derived from the last followup in November 1978.

The diagnostic criteria for multiple myeloma were the same as those outlined by a Committee of the Chronic Leukemia-Myeloma Task Force (7). Patients with marrow plasmacytosis, osteolytic skeletal lesions, and a single type of paraprotein in the serum or urine (or both) were considered for treatment.

Good response was defined as improvement in the following objective parameters: (*a*) a decrease of > 50% in the myeloma protein in serum and urine or a decrease to normal values, (*b*) an increase of at least 2 g/100 ml in the hemoglobin level and an increase to > 10 g/100 ml for at least 4 weeks in the absence of transfusion, (*c*) an increase to > 3 g/dl of serum albumin in those who at diagnosis had a smaller value, (*d*) a reduction of > 50% in palpable or roentgenographically demonstrated plasmacytomas, (*e*) a decrease of > 20% in the marrow plasma cells or a return to normal values, and (*f*) definitive radiographic evidence of skeletal healing (the size and number of lytic lesions could not have increased). Partial response was defined as the fulfillment of at least one of the above-mentioned conditions. No response was defined as the absence of response in any of the above-mentioned parameters.

Patients lost to followup within 3 months from the beginning of treatment were considered nonevaluable.

The clinical staging system described by Durie and Salmon (8) was employed to estimate body myeloma cell number.

Patients were divided into three groups according to their performance status at diagnosis: *good performance status* = patients leading a normal life without pain or with only slight pain; *average performance status* = ambulatory patients with moderate pain, weight loss, and anorexia; and *poor performance status* = bedridden patients with severe bone pain, fever, and anorexia which hindered ambulation.

The survival curves were calculated by the life-table method (9). The differences between curves were determined by the log-rank test (10).

TREATMENT GROUPS

Patients were randomly allocated without stratification to one of two regimens: *group A* = MeCCNU (100 mg/m^2 orally every 8 weeks), cyclophosphamide (600 mg/m^2 iv every 4 weeks), and prednisone (40 mg/m^2/day orally for 7 days every 4 weeks); *group B* = melphalan (8 mg/m^2/day orally for 4 days every 4 weeks) and prednisone (40 mg/m^2/day orally for 7 days every 4 weeks). Both groups received sodium phosphate (4 g/day orally), oxymetholone (25 mg/m^2/day orally), and calcium gluconate (2 g/day orally). Local radiation therapy for bone pain was permitted, and transfusions, antibiotics, and other supportive therapies were used as needed. Quantification of M protein in serum and urine was done every 3 months. Marrow aspiration and roentgenograms of all bones were done every 6 months.

RESULTS

Comparability of Treatment Groups

One hundred and forty-eight previously untreated patients with multiple myeloma were assigned at random to either group A (MeCCNU-cyclophosphamide-prednisone; 71 patients) or group B (melphalan-prednisone; 77 patients). Four and five patients from groups A and B respectively were lost to followup within 3 months, and 67 and 72 patients respectively were considered evaluable. The frequency of different parameters at diagnosis, such as age and type of M protein, was similar in both groups. The absolute tumor mass based on the clinical staging described by Durie and Salmon (8) showed a similar number of stage I patients in both groups, fewer stage II patients in group A, and more stage III patients in group A. As discussed later, there was no difference in survival between patients in stages II and III (table 1).

Therapeutic Response

Of 67 patients treated with MeCCNU-cyclophosphamide-prednisone, 32 (48%) had a good response, 18 had a partial response, and 13 failed to respond; four died within 3 months from the beginning of treatment but were considered evaluable. Of 72 patients treated with melphalan-prednisone, 24 (33%) achieved a good response, 25 achieved a partial response, 13 had no response, and ten died. Patients in group A had a slightly better response; however, this difference was not statistically significant ($P < 0.10$). The overall response (good plus partial responses) was 75% for group A and 65% for group B ($P < 0.30$) (table 2). The median time to best response in relation to the amount of M protein in serum and urine in those who achieved good response was 13.6

TABLE 1.—Comparability of patient groups

	Group A (MeCCNU-cyclophospha-mide-prednisone)	Group B (melphalan-prednisone)	Totals
No. of patients treated	71	77	148
No. lost to followup (< 3 mos)	4	5	9
No. evaluable	67	72	139
Median age (range)	59 (20–85)	59 (35–84)	
Clinical stage			
I	8	11	19
II	21	33	54
III	38	28	66
Renal function			
Creatinine level < 2.0 mg/100 ml (subtype A)	53	56	109
Creatinine level ⩾ 2.0 mg/100 ml (subtype B)	14	16	30
Immunoglobulin type			
IgA	13	17	30
IgG	35	40	74
IgD	1	—	1
Bence Jones protein (light chain)	10	12	22
Nonsecretory	1	1	2
Unknown	7	2	10
Performance status			
Good	25	31	56
Average	26	25	51
Poor	16	16	32

TABLE 2.—Therapeutic results according to treatment group

Group	No. of responses (%)			No. of deaths (< 3 mos)	Totals
	Good	Partial	None		
A (MeCCNU-cyclophosphamide-prednisone)	32 (48)	18	13	4	67
B (melphalan-prednisone)	24 (33)	25	13	10	72
Totals	56 (40)	43	26	14	139

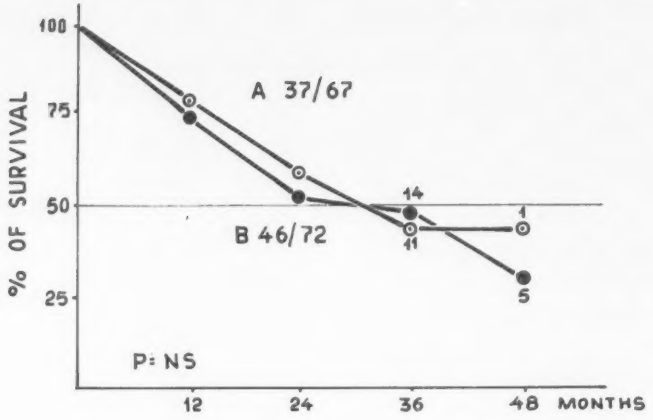

FIGURE 1.—Survival according to therapeutic schedule. The Nos. at each point = the patients at risk. The Nos. after "A" and "B" = the No. of deaths/total treated.

months (range, 5–35 months) in group A and 12.7 months (range, 4–35 months) in group B.

Survival

The survival curves for both groups of patients were similar. The median survival for all the patients from diagnosis was 32 months, with 42% of group A alive at 48 months and 30% of group B alive at 48 months (fig 1).

The analysis of survival according to therapeutic response shows a median survival of 48 months for patients with good response, and 24 of 56 patients died. The median survival was 22 months for partial responders, and 27 of 43 patients died. The median survival of those who failed to respond or died within 3 months from the beginning of therapy was 9 months, and 32 of 40 patients died. The difference between good versus partial response or partial versus no response was highly significant ($P < 0.0005$) (fig 2).

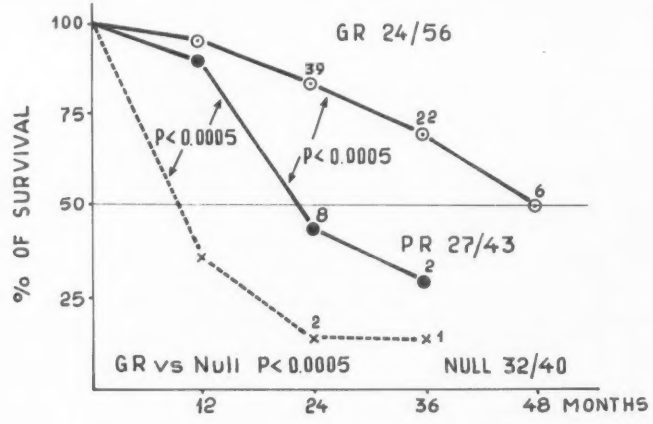

FIGURE 2.—Survival according to therapeutic response. GR = good response; PR = partial response; NULL = no response.

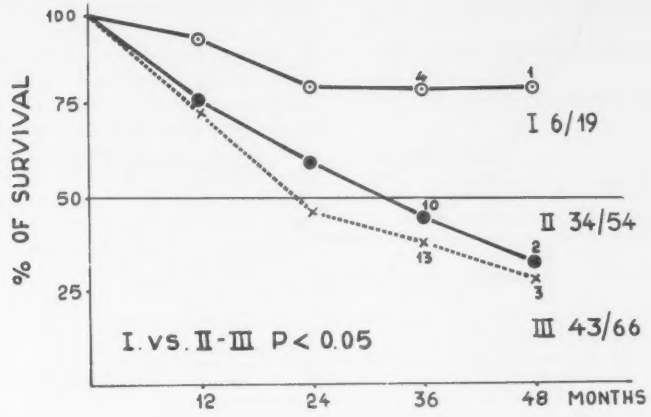

FIGURE 3.—Survival according to clinical stage (Durie and Salmon [8]).

Using the clinical staging system described by Durie and Salmon (8), of 19 patients who were in stage I at diagnosis, 80% remain alive at 48 months. The median survival was 32 months for 54 patients in stage II and 23 months for 66 patients in stage III. The survival of patients in stage I was significantly longer than that of patients in stages II and III ($P < 0.05$) (fig 3). However, the survival of patients in stage II versus patients in stage III was not significantly different. No statistically significant difference was observed in survival when comparing each clinical stage in groups A and B (table 3).

A total of 109 patients had good renal function at diagnosis (creatinine level < 2.0 mg/100 ml), subtype A of the classification of Durie and Salmon (8), and 62 died with a median survival of 34 months. Thirty patients had renal failure (creatinine level ≥ 2.0 mg/100 ml), subtype B, and 21 died with a median survival of 15 months. This difference was not significant (fig 4). Of those patients with renal failure, one was in stage I, eight were in stage II, and 21 were in stage III. The median survival was 23 months for patients in stage IIB and 14 months for those in stage IIIB. The survival of all patients was evaluated according to performance status at diagnosis (fig 5). Fifty-six patients with good performance status had a median survival of > 48 months, with 54% alive at that time. Fifty-one patients with average performance status had a median survival of 25 months, with 30% alive at 48 months. A total of 32 patients with poor performance status had a median survival of 12 months, and 13% were alive at 48 months. The statistical difference was $P < 0.001$ between the good and average groups, $P < 0.01$ between the average and poor groups, and $P < 0.0005$ between the good and poor groups.

Toxicity

All patients treated with MeCCNU and cyclophosphamide had some degree of nausea and/or vomiting caused by both drugs. No gastrointestinal toxicity was observed in the melphalan group. The median nadirs of leukocyte count, hemoglobin level, and platelet count were quite comparable in both groups. The median nadir was calculated 3 months after treatment and during the entire period in which the treatment was employed. The median nadir leukocyte count was 3220/mm³ (range, 1000–7000/mm³) for group A and 3100/mm³ (range, 1300–6100/mm³) for group B. The median nadir hemoglobin level was 9.7 g/100 ml (range, 5.9–13.0 g/100 ml) for group A and 10.0

TABLE 3.—Survival according to therapeutic schedule and clinical stage

	Median survival (mos)		% of patients surviving at 36 mos		
Stage	Group A	Group B	Group A	Group B	*P*.value*
I	46	>48	88	75	NS
II	28	38	35	53	NS
III	25	20	39	33	NS

*NS = not significant.

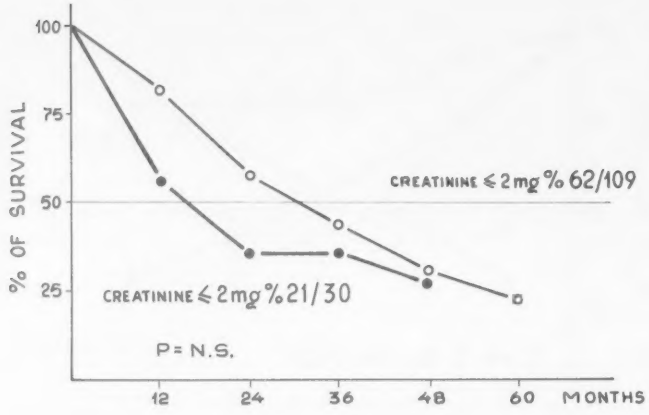

FIGURE 4.—Survival according to renal function.

g/100 ml (range, 6.5–13.7 g/100 ml) for group B. The median nadir platelet count was 160,000/mm³ (range, 40,000–280,000/mm³) for group A and 140,000/mm³ (range, 30,000–290,000/mm³) for group B. The amount of drug received in each course in both schedules was determined by the degree of leukopenia. In group A 37% of the patients had reductions in the amount of drugs in one or more courses compared to 49% in group B. Two patients developed erythroleukemia, which resulted in death at 42 and 73 months from diagnosis. Both were in group B.

DISCUSSION

This report summarizes the results of a randomized comparison of melphalan-prednisone versus MeCCNU-cyclophosphamide-prednisone in the treatment of previously untreated multiple myeloma. The data for 139 evaluable cases show no statistical differences in the percent of good response or overall response in both treatment regimens. Also, the median survival of 32 months was the same in both schedules.

Alexanian et al (1) was the first to report a greater response in patients treated with melphalan-prednisone compared to melphalan alone. This was confirmed by Costa et al (11) from the Acute Leukemia Group B in patients with "good risk" where prednisone-melphalan was significantly better than melphalan alone; however, in poor-risk patients the combination failed to produce more frequent responses and was associated with a shorter survival.

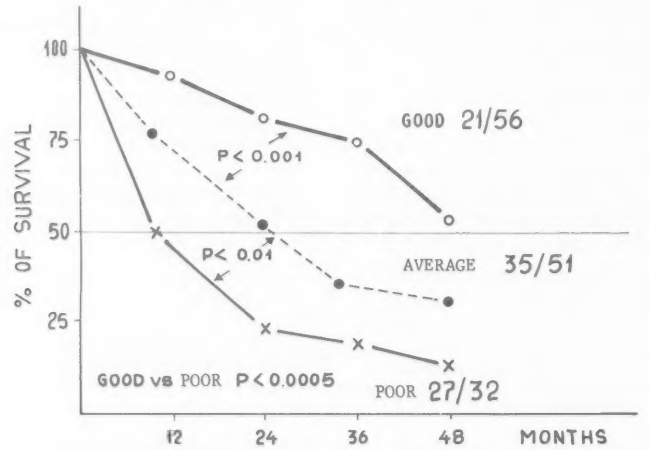

FIGURE 5.—Survival according to performance status at diagnosis.

Lee et al (5) found that the combination of low doses of BCNU and vincristine with moderate doses of melphalan, cyclophosphamide, and prednisone produces a response rate and survival time higher than those resulting from previous trials using melphalan-prednisone. Alexanian et al (4) compared six different chemotherapy regimens in 462 previously untreated patients. The drug combination that included vincristine was associated with a higher response rate and a longer survival. None of the groups received melphalan plus prednisone alone. Bergsagel et al (6) reported the results in 320 previously untreated patients who received three drug combinations. One group received melphalan-prednisone, another received melphalan-prednisone-cyclophosphamide-BCNU in a sequential schedule, and the third received prednisone-melphalan-cyclophosphamide-BCNU given together. No significant difference in the response rate or the median survival was found among the three treatment groups.

A comparison of the survival according to response shows a marked difference and is probably the most important test to measure tumor regression. At 36 months, 70% of those who achieved a good response were alive, 29% of those who achieved a partial response were alive, and 13% of those with no response were alive.

We employed the clinical staging system used by Durie and Salmon (8). These criteria define patients with high, intermediate, and low numbers of total body myeloma cells. At 48 months, 80% of those in stage I were alive, 33% of those in stage II were alive, and 28% of those in stage III were alive. There was a statistical difference between stage I versus stages II and III ($P < 0.05$). This study confirms the adequacy of this staging system in measuring prognosis and the excellent prognosis of patients in stage I, and emphasizes the usefulness of stratifying patients according to clinical stage in controlled clinical trials. Also, the performance status at diagnosis shows real value as a prognostic factor in the evaluation of survival.

We conclude that treatment of multiple myeloma with MeCCNU-cyclophosphamide-prednisone is not more effective than treatment with melphalan-prednisone.

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Synchronization of L1210 Leukemia With Hydroxyurea Infusion and the Effect of Subsequent Pulse Dose Chemotherapy[1,2]

Ruth E. Moran* and Marc J. Straus[3,4]

SUMMARY

Studies were performed to synchronize L1210 tumor cells in S phase in an effort to maximize the effect of subsequent pulse dose chemotherapy. Hydroxyurea (HU) was administered by continuous iv infusion 5 days after tumor implantation. Perturbation effects on the S-phase cells were measured by serial tritiated thymidine labeling indices. Effects on cell-cycle progression were measured by DNA content distribution analysis using flow cytometry. Synchronization of tumor cells was achieved with HU infusion (48 mg/kg/hour × 24 hours), resulting in 90% of the cells in S phase. Following infusion, synchronous progression of S-phase cells into G_2/M and then G_1 was apparent from +0 to +10 hours later. The susceptibility of HU-synchronized cells to subsequent chemotherapy was determined by treating mice with cytosine arabinoside (Ara-C), methotrexate (MTX), or Adriamycin (ADR) pulse doses at various intervals following infusion. Synergy, measured by prolongation of survival times, resulted when Ara-C was administered immediately after the end of the infusion. Survival times then decreased as the fraction of cells in S phase decreased. In contrast, the survival times of mice treated with MTX or ADR pulse doses after HU infusion were additive at best and did not correlate with fluctuations in the S phase compartment. Therefore, prior synchronization of tumor cells in S phase was therapeutically advantageous when coupled with appropriately timed Ara-C pulse doses. There was little advantage in combining HU infusion with subsequent MTX or ADR therapy.

[Cancer Treat Rep 64:81–86, 1980]

Progression delay of tumor cells in a discrete phase of the cell cycle has been demonstrated for a variety of anticancer drugs (1–3). Subsequent intervention with a second phase-specific cytotoxic agent at the appropriate time interval may result in an increased therapeutic benefit if the percentage of cells at risk is increased. A critical parameter, therefore, is whether cells blocked by the first agent are capable of further proliferation. This is an important consideration in the design of chemotherapy schedules aimed at synchronizing cells in an effort to render the population more susceptible to subsequent chemotherapy. If the synchronizing agent also kills the synchronized cells, then subsequent therapeutic intervention may be of little value.

In a previous publication we characterized in detail the dose response of L1210 tumor cells to continuous iv infusion of hydroxyurea (HU) (4). Using flow cytometry (FCM), it was demonstrated that a 24-hour infusion of HU (48 mg/kg/hour) effectively synchronized cells in S phase with little or no cytotoxicity. After infusion, cell-cycle transit was rapidly re-established and cells synchronized in S phase progressed into G_2/M and then G_1 within a 10-hour period.

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[4]We wish to acknowledge Susan Fish for excellent technical assistance.

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This report represents a continuation of these studies. Serial tritiated thymidine (^{3}H-TdR) labeling indices (LI) and FCM distributions have been determined for tumor and normal bone marrow cells at frequent intervals following HU infusion. The susceptibility of HU-synchronized cells to a subsequent pulse dose of chemotherapy, either cytosine arabinoside (Ara-C), methotrexate (MTX), or Adriamycin (ADR), has been determined and related to the cell-cycle kinetics of these target-cell populations. Schedule-dependent effects have been measured by varying the interval between the infusion and the pulse dose and measuring the host survival time.

MATERIALS AND METHODS

Mice.—Male BALB/c × DBA/2 (hereafter called $CD2F_1$) mice were purchased from Lab Supply Co, Indianapolis, IN. Male DBA/2 mice were purchased from Charles River Breeding Laboratories, Wilmington, MA.

L1210 leukemia cells.—L1210 ascites tumor was obtained from the Mason Research Institute, Worcester, MA, and maintained in our laboratory by weekly passage in male DBA/2 mice. Experiments were performed on $CD2F_1$ mice following ip implantation of 10^5 tumor cells on Day 0.

Drugs.—HU, Ara-C, and MTX were supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. ADR was supplied by Adria Laboratories, Columbus, OH. HU, administered by continuous iv infusion, was diluted in a sterile 0.9% NaCl solution containing heparin (10 units/ml) to a final concentration of 48 mg/kg/hour based on the mean body weight of the mice on the day of treatment. The infusion rate was 1 ml/24 hours. Ara-C, MTX, and ADR were given by ip pulse injection. Ara-C and ADR were diluted in a sterile 0.9% NaCl solution to a final concentration of 0.01 ml/g of body weight. MTX was diluted in a sterile 0.9% NaCl solution, titrated with NaOH to pH 7.5, and injected at a final concentration of 0.01 ml/g of body weight.

Experimental design.—$CD2F_1$ mice were injected ip on Day 0 with 10^5 tumor cells diluted to a total volume of 0.2 ml with Hanks' balanced salt solution. Five days later the mice were randomized to receive: (*a*) no treatment; (*b*) HU infusion; (*c*) Ara-C, MTX, or ADR single pulse dose; or (*d*) HU infusion followed by a single pulse dose of Ara-C, MTX, or ADR at +0 to +24 hours after infusion. HU infusion (48 mg/kg/hour × 24 hours) was started on Day 5. After the infusion separate groups of mice (four mice per group) were given injections of 20 microcuries of ^{3}H-TdR (New England Nuclear Corp, Boston, MA) at +0, +3, +7, +10, or +24 hours, and 1 hour later the mice were killed and tumor and bone marrow cells were collected and processed for LI and FCM analysis. At the same time intervals, separate groups of HU-treated mice (12 mice per group) received a single pulse dose of Ara-C (20 mg/kg), MTX (20 mg/kg), or ADR (5 mg/kg) and survival times were determined. Survival times of these mice were then compared to the survival times of untreated tumor-bearing control mice or mice treated with each agent given independently. Earlier studies showed that when given as a single pulse dose on Day 5 or 6, these doses are equicytotoxic and increase host survival by approximately 1 day. The Day-6 data are presented.

LI determinations.—Labeled L1210 cells were collected and prepared for autoradiography as previously described (5). The slides were dip-coated in Kodak NTB2 liquid emulsion (Eastman Kodak, Rochester, NY), exposed for 6 days, developed in Kodak D-19 developer, and fixed in Kodak Ektaflo fixer. Autoradiographic background averaged < 1 grain/nucleus. Nuclei with ≥ 3 grains were considered labeled. One thousand cells were scored for LI determination.

FCM determinations.—Cells were stained with propidium iodide according to the method of Krishan (6). Cells were analyzed using a model 4800A Cytofluorograf and 2102 Pulse Height Analyzer (Ortho Instruments, Westwood, MA). One hundred thousand L1210 cells or 250,000 bone marrow cells were analyzed for each histogram. Machine conditions were controlled using untreated L1210 cells and/or mouse peripheral blood as an external standard. Machine drift was ±2 channels.

RESULTS

FCM and LI Studies

Mice received HU infusions (48 mg/kg/hour × 24 hours) beginning on Day 5 of tumor growth. Cytokinetic parameters of tumor and normal bone marrow cells were then measured during the first 24 hours after infusion. The serial FCM histograms of the L1210 cells are shown in figure 1. There was little variation in histograms from the separate mice per group and, therefore, only one histogram per time point is shown. Detailed LI data are shown in figure 2. LI represent the mean of four mice per group.

FCM analysis of cells from untreated control mice was identical on Days 5 and 6, whereas the LI decreased slightly from 65% to 60%. The control histogram shown in figure 1 was obtained on Day 6 and corresponds to the time the infusion was ended. Each of the remaining histograms represents cells collected from HU-treated mice from +0 to +24 hours after infusion. It is apparent from these data that HU infusion effectively synchronizes cells in S phase and that the effects are reversible. At the end of the infusion (+0 hour) most of the cells are in the S region of the histogram and 90% of the cells are labeled (figs 1 and 2). Most of the remaining cells appear to be in G_2. As the interval after infusion increases, cell transit

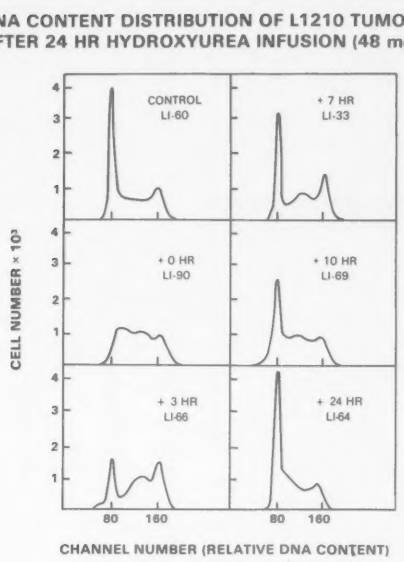

FIGURE 1.—L1210 relative DNA content distribution as a function of time after HU infusion. Each histogram represents 100,000 cells. Corresponding LI are indicated on each panel.

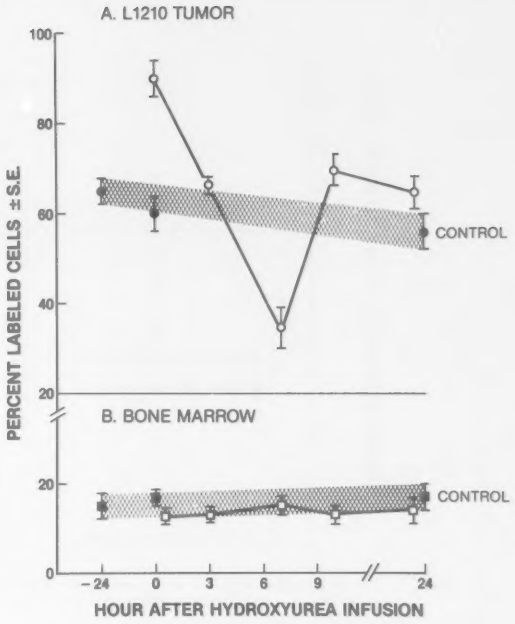

FIGURE 2.—LI as a function of time following HU infusion. Solid symbols represent LI of untreated cells; corresponding open symbols represent LI at different time intervals after infusion.

through S to G_2/M is apparent and this is accompanied by a decrease in the LI from 90% to 33% within 7 hours. Cell division with repopulation of the G_1 peak is apparent from +3 to +7 hours. The LI then increased to 69% at +10 hours as the semisynchronized cohort of G_1 cells progressed into S phase. By +24 hours normalization of values occurred.

In contrast to the effects on the tumor cell population only minor changes were observed in the bone marrow. There was no evidence of progression delay of cells in S phase on FCM analysis (data not presented). The LI did not vary significantly from the control of 15% ± 3% (fig 2).

Survival Studies

The survival times of mice treated with HU infusion followed by Ara-C (20-mg/kg pulse dose) given at intervals from +0 to +10 hours later are shown in figure 3. Untreated tumor-bearing control mice lived 8.3 days. Mice treated with HU infusion alone lived an additional 1.1 days. Ara-C (20 mg/kg) resulted in a 0.9-day increase in lifespan. The estimated additive effect is the sum of the increases in lifespan above the control value for each of the drugs given independently (8.3 + 1.1 + 0.9 = 10.3 days). When Ara-C was given immediately after HU infusion (+0 hour), the mice lived 1 day longer than the expected additive effect. Survival times then decreased as the interval between HU and Ara-C increased. The decrease in survival times coincided with a depletion of the S-phase compartment observed on FCM (fig 1). The average survivals of the +7- and +10-hour pulse schedule groups were slightly less than additive. The mean survival of the infusion plus Ara-C +0-hour group was significantly longer than that of each of the other three infusion plus pulse groups ($P < 0.05$).

The survival times of mice treated with HU infusion plus MTX pulse dose at intervals from +0 to +24 hours later are shown in figure 4. There were no differences in the survival times of mice treated with MTX from +0 to +10 hours after HU infusion. The effects of these drug schedules were additive. A slight increase in survival was observed when the MTX was delayed for 24 hours.

The survival times of mice treated with HU infusion plus ADR pulse dose at intervals from +0 to +24 hours later are shown in figure 5. As with MTX, the effects of HU plus ADR were additive when ADR was given within 10 hours after infusion. ADR given 24 hours later was less than additive.

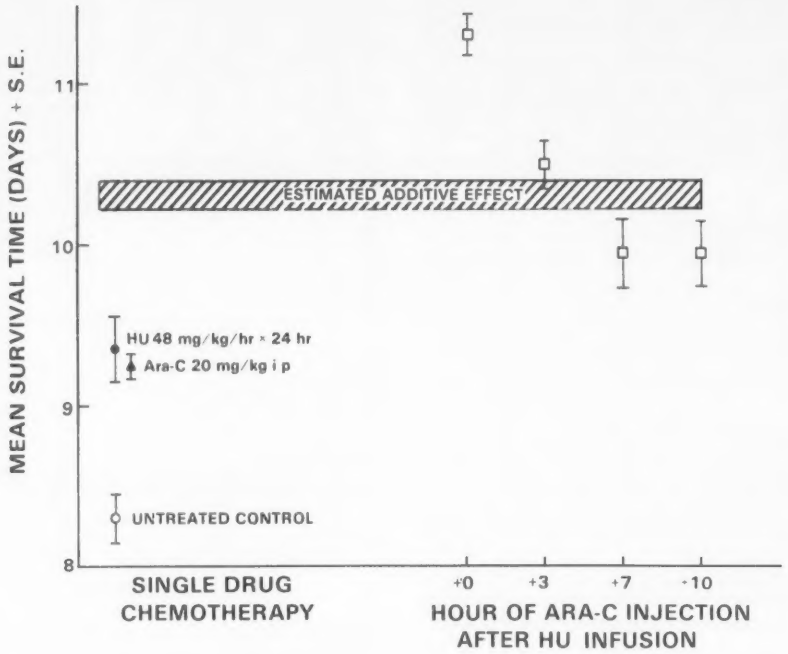

FIGURE 3.—Survival times of untreated tumor-bearing mice and mice treated with HU infusion alone, Ara-C pulse dose alone, or the combination. The estimated additive effect is based on the sum of the increases above control of the drugs given independently. Each point represents the mean of 12 mice.

DISCUSSION

These studies indicate that manipulation of the cell-cycle kinetics of tumor cells in vivo may be therapeutically advantageous when coupled with an effective second agent given at the appropriate time interval. HU infusion followed by Ara-C pulse was synergistic when Ara-C was given immediately after the infusion, at the time when most cells were synchronized in S phase and actively synthesizing DNA.

The 1-day increase in survival time observed after Ara-C alone on Day 6 is consistent with a cell kill of approximately 60% for a population with a doubling time of 13–18 hours (7), and is consistent with an LI of 60%. The 1-day increase in survival following HU infusion is primarily due to progression delay effects rather than cell kill (4). When Ara-C was administered at the time of maximum S-phase synchrony (+0 hour after infusion), there was a 3-day increase in survival. This is equivalent to a 1-day increase in survival due to the HU infusion and a 2-day increase which would occur if 90% of the tumor cells were killed by the pulse dose of Ara-C. A 90% cell kill is consistent with the +0-hour LI of 90%. The decrease in survival time after longer intervals between infusion and pulse dose correlated with a decrease in LI. The data therefore suggest that the greater the degree of S-phase synchrony, the greater the cell kill that can be achieved with the Ara-C pulse.

These results are in contrast to the effects of HU infusion followed by a single pulse dose of MTX or ADR where survival times were not significantly different from the expected additive effect of the drugs given independently. The effect of these drugs was not schedule-dependent and did not correlate with the fluctuation in the S-phase compartment observed on FCM. The lack of synergy with MTX may be related to the mechanism of action of MTX rather than specific cytokinetic effects. As with Ara-C, MTX inhibits DNA synthesis and is classified as S-phase specific. However, MTX acts by inhibiting dihydrofolate reductase which in turn interferes with de

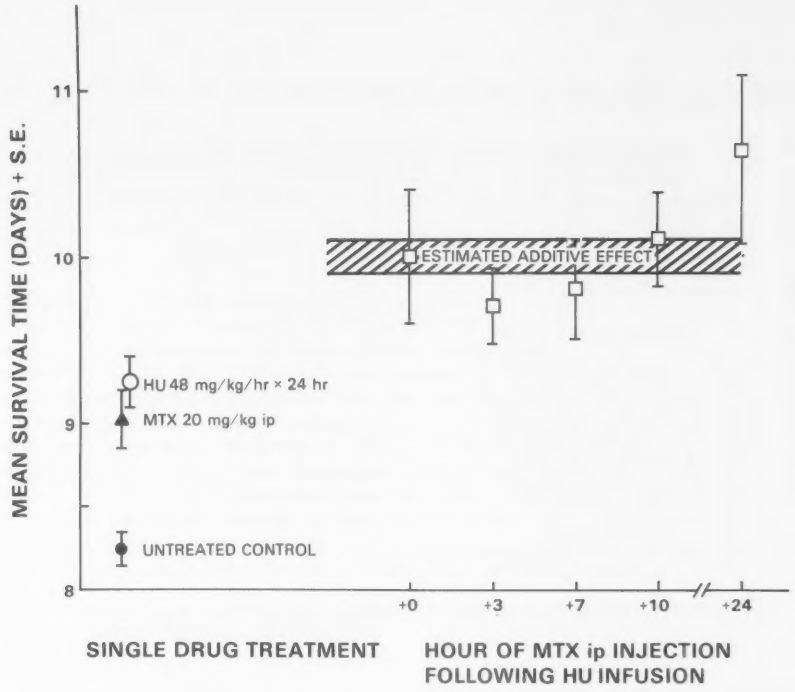

FIGURE 4.—Survival times of untreated tumor-bearing mice and mice treated with HU infusion alone, MTX pulse dose alone, or the combination. The estimated additive effect is based on the sum of the increases above control of the drugs given independently. Each point represents the mean of 12 mice.

novo synthesis of thymidylate (8). Under the conditions of this study, cells were slowed in S phase for a 24-hour period prior to MTX exposure. The effects of noncytotoxic infusion doses of HU on the de novo production of DNA precursors has not been measured. If the concentration of thymidylate increases during this period, then the effect might be to lower the cytotoxic potential of MTX. In contrast, Ara-C acts by inhibiting DNA polymerase activity. Changes in the nucleotide pool sizes would have less effect on the Ara-C activity. The lack of detectable schedule-dependent effects may also be due to a delayed onset of action of MTX.

Recent data of Göhde et al (1) have shown that ADR induces progression delay of cells in G_2. However, the drug effectively kills cells in all phases of the cell cycle. These data, together with the results presented here, suggest that there may be no particular advantage to treating with ADR following cell-cycle synchronization. Other doses of MTX and ADR need to be tested for further confirmation.

The influence of bone marrow or gastrointestinal toxic effects on the survival of treated mice was not measured. However, the survival times and the pattern of weight changes for the treated mice would suggest that deaths were due to disease progression and not toxic effects.

There have been no other reported studies of HU infusion coupled with other phase-specific pulse therapy. However, synchronization of tumor cells in S phase by intra-ascitic infusion has been demonstrated by Hartenstein et al (9). The results presented here demonstrate the potential therapeutic benefit of cell-cycle synchronization prior to appropriately timed phase-specific pulse therapy.

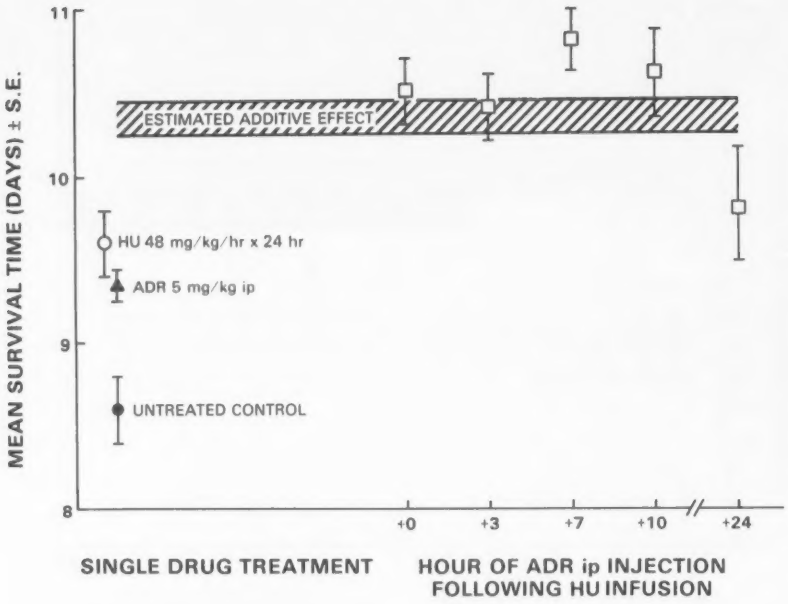

FIGURE 5.—Survival times of untreated tumor-bearing mice and mice treated with HU infusion alone, ADR pulse dose alone, or the combination. The estimated additive effect is based on the sum of the increases above control of the drugs given independently. Each point represents the mean of 12 mice.

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A Role of *cis*-Dichlorodiammineplatinum(II) in Squamous Cell Lung Cancer[1,2]

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SUMMARY

Forty-one patients with advanced squamous cell lung cancer and no prior chemotherapy were entered in a prospectively randomized trial comparing dianhydrogalactitol plus Adriamycin (DA) versus DA plus *cis*-dichlorodiammineplatinum(II) (DAP). The DAP regimen was superior to the DA regimen in regression rate (53% versus 27%), median regression duration (255 versus 122 days), median time to tumor progression (approximately 175 versus 58 days), and median survival time (185 versus 126 days). Patients who were > 60 years old responded particularly well to the DAP regimen and accounted for most of the survival advantage. Nausea, vomiting, and myelosuppression were more frequent and severe with the DAP regimen. This study seems to indicate a role of *cis*-dichlorodiammineplatinum(II) in patients with advanced squamous cell lung cancer. The particular advantage noted for older patients needs further evaluation.

[Cancer Treat Rep 64:87–91, 1980]

We have previously reported on the antitumor effects of both dianhydrogalactitol (DAG) and the combination of cyclophosphamide (CYC), Adriamycin (ADR), and *cis*-dichlorodiammineplatinum(II) (CDDP) (CAP-I) in patients with advanced non-small cell lung cancer (1). In patients with squamous cell lung cancer and no prior chemotherapy, DAG had a regression rate of 15% (four of 26 patients) and CAP-I had a regression rate of 44% (eight of 18).

Because the regression rate for DAG in patients with squamous cell lung cancer in our previous study was certainly comparable to the 4% regression rate for CYC in the randomized trial reported by Bodey et al (2), and because it has less emesis and alopecia associated with its use, we decided to substitute DAG for CYC in the CAP-I combination resulting in DAP (DAG, ADR, plus CDDP).

The exact nature of the role of CDDP in the CAP-I regimen is uncertain. We have hypothesized (1) that the efficacy of the regimen was the result of multiple two-drug synergistic relationships (CYC plus ADR, CYC plus CDDP, and ADR plus CDDP). The results of our phase II evaluation of CDDP as a single agent in adenocarcinoma of the lung (3) indicated a poor regression rate of 9% (two of 22 patients) that did not seem to explain the high regression rate of the combination. Rossof et al (4) could also document little single-drug activity for CDDP; they had a regression rate of 5% (two of 37 patients). In the present study, we elected to evaluate the role of CDDP, not as a single agent, but rather as an integral part of a drug combination. This randomized trial reported here was therefore designed to allow evaluation of two new drug combinations (DA [DAG plus ADR] and DAP) as well as the role of CDDP in the DAP combination.

STUDY DESIGN AND TREATMENTS

A prospectively randomized study was conducted. An informed written consent was obtained from all patients prior to entry in the study. After stratification, patients were randomized to receive either the two-drug regimen, DA, or the three-drug regimen, DAP. Patients progressing with DA received the two-drug combination of CYC

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plus CDDP. Patients who progressed with DAP were removed from the study.

The doses of DAG and ADR were identical in the two regimens and consisted of 25 mg/m²/day × 3 days iv of DAG plus 40 mg/m² iv on Day 1 of ADR. The DAP group received 40 mg/m² iv on Day 1 of CDDP given as a rapid iv infusion over 5–10 minutes without special hydration or diuretics. All treatments were scheduled to be repeated 4 weeks from Day 1 with subsequent treatments given every 4–5 weeks. The maximum permissible total cumulative dose of ADR was 500 mg/m². In the CYC plus CDDP regimen, the dose of CYC was 300 mg/m²/day × 3 days iv and the dose of CDDP was 15 mg/m²/day × 3 days iv with the same re-treatment schedule as given above.

The doses of DAG and ADR were increased and decreased to obtain wbc count nadirs of between 1500 and 2500/mm³ and/or platelet count nadirs of between 75,000 and 100,000/mm³. Doses of CDDP were adjusted based on the serum creatinine level at the time of re-treatment: 30 mg/m² with creatinine levels of 1.3 or 1.4 mg/dl and 20 mg/m² with creatinine levels of between 1.5 and 1.9 ml/dl; CDDP was permanently discontinued if the creatinine level was ≥ 2.0 mg/dl.

Patients

Forty-four patients with advanced squamous cell lung cancer were entered in the study. Twenty-two of these patients were randomized to receive DA and the other 22 were randomized to receive DAP. Three patients who were randomized to receive DAP were subsequently found to have a non-squamous histology (two with large cell and one with adenocarcinoma) and were declared to be ineligible. Among these three patients, two had tumor regressions and one had tumor progression. Therefore, final analysis was performed for 22 patients who received DA and for 19 who received DAP.

Patients were stratified according to Eastern Cooperative Oncology Group (ECOG) performance scores (0–1 versus 2–3) and the presence of central nervous system (CNS) metastases (yes versus no). Descriptive factors included age and sex. The distribution of each of these co-variates and other pretreatment characteristics by treatment is presented in table 1. Six of the seven patients presenting with CNS metastases were treated with corticosteroids and CNS irradiation immediately prior to, or concomitantly with, the start of chemotherapy; one patient received neither treatment. The overall median age was 60 years (range, 38–75); the median age of the DA group was 64 years (range, 41–75) and the median age of the DAP group was 57 years (range, 38–69). A significant imbalance existed between age and treatment, with 14 of 19 of the patients (74%) receiving DAP being < 60 years old compared to only six of 22 of the patients (27%) receiving DA being < 60 years old. Other co-variates were well-balanced between treatments, although the DAP group did have a somewhat higher proportion of females than the DA group (32% versus 14%).

TABLE 1.—Pretreatment characteristics of the 41 evaluable patients

Characteristic	Treatment regimen	
	DA	DAP
No. of evaluable patients	22	19
Median age in yrs (range)	64 (41–75)	57 (38–69)
Sex (male:female)	19:3	13:6
No. of patients with—		
ECOG performance score (0,1:2,3)	14:8	12:7
Prior treatment of primary tumor		
None	14	11
Surgery	5	4
Irradiation	2	2
Surgery + irradiation	1	2
CNS metastases (yes:no)	3:19	4:15

RESULTS

Response Rates, Durations, and Time to Progression

Different response definitions were used depending on whether the patient had measurable disease or visible and evaluable, but not measurable, disease. For patients with measurable disease, standard definitions of complete regression, partial regression, stable disease, and disease progression were employed. For patients with evaluable disease, complete regression was the same as for measurable disease. Regression was defined as the unequivocal shrinkage of tumor; stable disease was defined as no definite regression or progression of tumor; and disease progression was defined as a definite increase in tumor size or the appearance of new lesions. All responses of evaluable disease required an agreement by two observers. The details and justifications for these definitions are explained in detail elsewhere (5). Tumor regressions included complete and partial regressions in measurable disease and regressions in evaluable disease.

Objective tumor regressions were noted in ten of 19 patients (53%) treated with DAP and in six of 22 (27%) treated with DA. Comparison of these two proportions via the two-sided Pearson chi-square statistic yielded a P value of 0.097. Interestingly, all seven patients receiving DAP who were at least 58 years old experienced regressions. There were no complete regressions among patients receiving DA whereas there were two complete regressions among 19 patients (11%) receiving DAP. One complete regression occurred after one course of DAP.

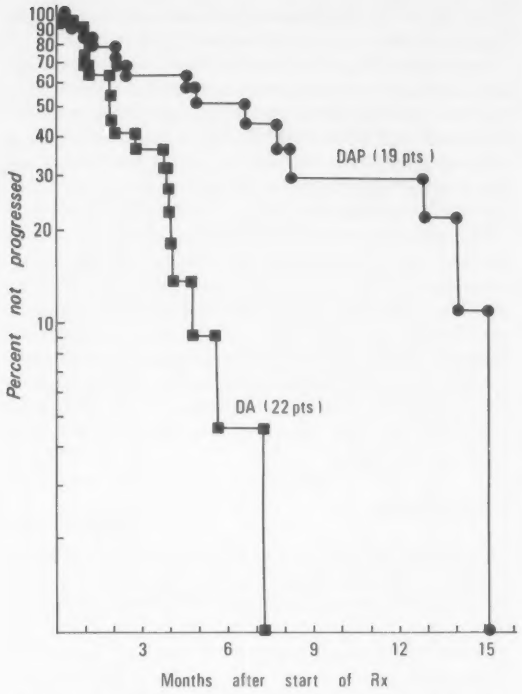

FIGURE 1.—Time to disease progression curves for DA and DAP.

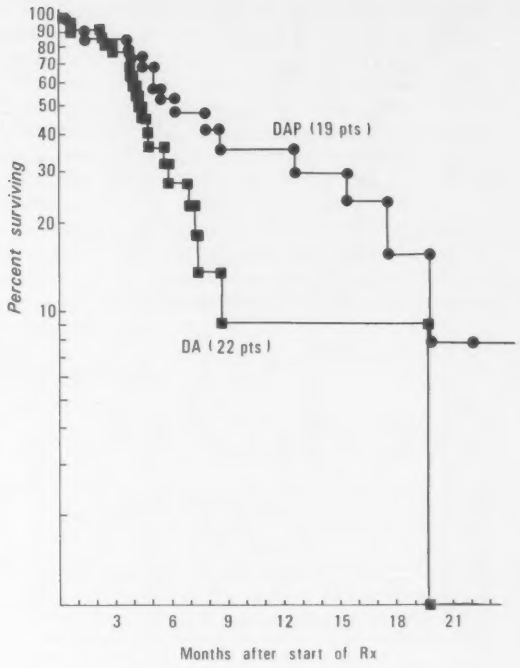

FIGURE 2.—Survival curves for patients treated with DA and DAP.

The patient refused further treatment and died 254 days following the first course. The other complete regression (bilateral pulmonary, supraclavicular, and CNS metastases) lasted for 421 days in the CNS (subsequently irradiated) and persists for > 525 days for the non-CNS disease (the last 225 days receiving no chemotherapy). DAP had to be discontinued in this patient because of severe and prolonged myelosuppression despite 75% dose reductions.

The durations of the DAP tumor regressions (calculated from Day 1 of treatment) were significantly longer ($P = 0.001$, log-rank [6]) than those of DA, with median durations of 255 and 122 days respectively.

Initial treatment with DAP was also found to delay the onset of tumor progression significantly longer than DA (fig 1). All curves are generated via the Kaplan-Meier technique (7). Gehan-Wilcoxon (8) and log-rank two-sided test procedures yielded P values of 0.011 and 0.002 respectively. The median time to tumor progression was 58 days for DA versus between 147 and 199 days for DAP. Using these procedures and a Cox analysis (9), no association was detected between time to progression and any of the co-variates of age, sex, performance score, or presence of CNS metastases ($P > 0.50$ for all tests). Not surprising then was the fact that the measure of the association between treatment and time to progression was unaffected when adjustment was made for each of the covariates via the Cox model.

Upon tumor progression, nine patients receiving DA initially were subsequently treated with CYC plus CDDP, with one partial regression noted. Two other patients, after being removed from the study because they did not meet cross-over eligibility, were treated with triazinate with no regressions noted. Six of the 19 patients receiving DAP were offered additional chemotherapy (two with maytansine, two with triazinate, one with *N*-phosphonacetyl-L-aspartic acid, and one with indicine-*N*-oxide), with no further tumor regressions noted.

Survival

Analysis was performed to determine whether patients receiving CDDP in addition to DAG and ADR would experience longer survival than those receiving only DAG and ADR initially, with CDDP and CYC upon progression. It should be noted that one might expect to find survival differences of less magnitude than would be seen if patients receiving DA initially were, like the patients

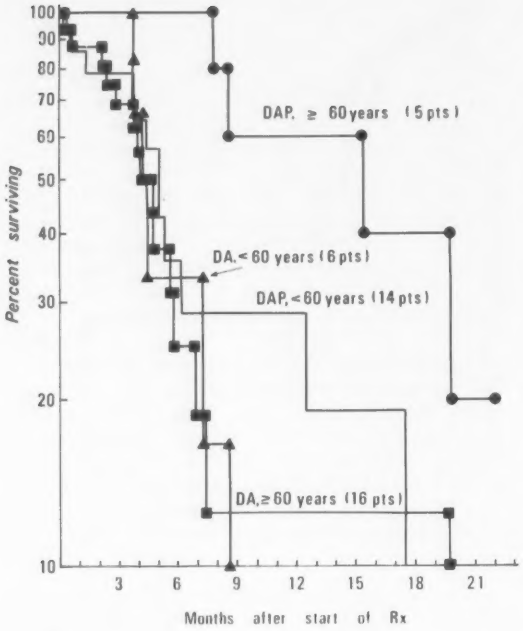

FIGURE 3.—Survival curves for patients treated with DA and DAP with patients in each regimen divided into 2 groups by age (< 60 vs ≥ 60 yrs).

receiving DAP, removed from the study at the first sign of tumor progression and not exposed to CDDP.

Gehan-Wilcoxon and log-rank two-sided test procedures and a Cox analysis detected no association between survival and any of the co-variates sex, performance score, or presence of CNS metastases ($P > 0.50$ for all tests). Hence, each of these co-variates, well-balanced with respect to treatment, was excluded from further survival analyses.

Using either a Cox or O'Brien two-sided test procedure (10), no association was detected between age and survival ($P = 0.90$) for patients who received DA. However, older patients receiving DAP survived significantly longer than younger patients ($P < 0.01$). Thus, a significant interaction exists ($P = 0.05$ by Cox model) between age and treatment, ie, the association between age and survival was specific to treatment.

Survival of the 19 patients who received DAP and the 22 patients who received DA is shown in figure 2. Gehan-Wilcoxon and log-rank two-sided test procedures yielded P values of 0.1176 and 0.676 respectively. The higher Gehan-Wilcoxon P value reflects the procedure's relative insensitivity in detecting differences occurring only later in time. The median survival was 185 days (range, 10–702+ days) for DAP-treated patients versus 126 days (range, 5–286+ days) for DA-treated patients.

In view of the previously noted imbalance between age and treatment and the previously established interaction between age and treatment (ie, the association between treatment and survival is specific to age), we feel it is important to inspect the association between treatment and survival separately for younger and then for older patients rather than for all ages combined.

If age is dichotomized at the median for all patients in the study (< 60 versus ≥ 60 years), there is no substantial association between treatment and survival among the patients < 60 years old (Gehan-Wilcoxon and log-rank two-sided P values of 0.8355 and 0.4816 respectively). However, as shown in figure 3, older patients (≥ 60 years old) receiving DAP survived significantly longer than the older patients (≥ 60 years old) receiving DA (Gehan-Wilcoxon and log-rank two-sided P values of 0.0055 and 0.0201 respectively).

Toxic Effects

The major subjective toxic effects from both regimens consisted primarily of nausea and vomiting. Ninety-two percent of the patients receiving DAP experienced these toxic effects which were severe in 62%. Only 52% of the patients receiving DA experienced nausea and vomiting which were severe in only 16%. Both regimens caused mild diarrhea during treatment in about 25% of patients. DAP produced somewhat more mild to moderate stomatitis (25% versus 8%).

As with the subjective toxic effects, DAP produced more frequent and significant myelosuppression, particularly thrombocytopenia (platelet count nadir < 130,000/mm^3). Following the first course of chemotherapy at full dose for all patients, leukopenia (wbc count nadir < 4000/mm^3) occurred in 83% of patients receiving DAP (median wbc count nadir, 2500/mm^3; range, 900–3500/mm^3) versus 71% receiving DA (median wbc count nadir, 2900/mm^3; range, 900–3900/mm^3). Also, following the first course of chemotherapy, thrombocytopenia occurred in 61% of patients receiving DAP (median platelet count nadir, 78,000/mm^3; range, 8000–110,000/mm^3) versus only 24% receiving DA (median platelet count nadir, 75,000/mm^3; range, 33,000–118,000/mm^3). With subsequent dose escalations and de-escalations, leukopenia of a comparable degree occurred in 94% of both treatment groups. DAP, however, still resulted in a more frequent incidence of thrombocytopenia than DA (83% versus 39%). It appears that CDDP, even at the relatively small dose of 40 mg/m^2, does contribute to myelosuppression, particularly thrombocytopenia. One patient receiving DAP died at 10 days from sepsis and bleeding with a wbc count of 900/mm^3 and a platelet count of 8000/mm^3.

Renal toxicity with the DAP regimen was infrequent and mild. Eighteen of the 19 patients treated with DAP

started with a normal serum creatinine level (≤ 1.2 mg/dl), with one patient having an initial serum creatinine level of 1.4 mg/dl. Six of the 18 patients (33%) with normal initial creatinine levels had some increase above baseline, but the highest serum creatinine level recorded was 1.5 mg/dl. All six patients had a return of the serum creatinine level to or toward normal with reduction in the CDDP dose, and at the time of this report, only four of 18 patients (22%) had an elevated creatinine at their last determination (all four having values of 1.3 mg/dl). The one patient who initially had a serum creatinine level of 1.4 mg/dl continued to have that same level.

The ADR was stopped in one patient receiving DAP at a cumulative dose of 130 mg/m^2 when the patient developed severe cardiorespiratory problems. That patient was felt to have had both right- and left-sided heart failure and severe chronic obstructive pulmonary disease. ADR might have played a role. No other cardiac problems were noted.

Alopecia was noted in all patients.

DISCUSSION

This study was designed with two principal goals in mind. The first goal was to evaluate the role of CDDP in squamous cell lung cancer when used as part of a multiple-drug combination, not as a single drug. As a single drug, both Britell et al (3) and Rossoff et al (4) found CDDP to have little activity in lung cancer, although only one case of squamous cell type was treated. The three-drug regimen (DAP) was superior to the two-drug regimen without CDDP (DA) with regard to time to disease progression, regression rate, regression duration, and survival. This strongly suggests to us a role for CDDP used in combination with DAG and ADR in squamous cell lung cancer. Our previous study of CAP (I) (1) also suggested considerable activity for a three-drug regimen including CDDP. The two-drug regimen of ADR and CDDP reported by Bonomi et al (11) obtained a regression in only one of seven patients with squamous cell lung cancer. Likewise, the two-drug regimen of hexamethylmelamine and CDDP reported by Krauss et al (12) obtained a regression in only one of 19 patients of that cell type treated. In this current study, we noted only one regression among nine patients treated with CYC and CDDP.

Our second goal was to evaluate DAP and DA so that we might get some idea of their relative efficacy vis-a-vis CAP (I) and CYC plus ADR. The 53% regression rate for DAP was somewhat higher than the 44% regression rate noted for CAP, but we do not wish to declare any superiority for DAG over CYC since we feel that the difference is not sufficiently large to make that extrapolation without using a prospectively randomized trial. The 27% regression rate for DA is higher than the 4% regression rate obtained by the Veterans' Administration Lung Cancer Study Group (13) for CYC plus ADR, their best regimen to date. Again, we do not wish to make any extrapolations. A study comparing DAP versus CAP in a prospectively randomized fashion is in progress.

The apparent synergistic effect from the addition of an alkylating agent (DAG or CYC) to the combination of ADR and CDDP appears real, but demands further testing. Recently, Takita et al (14) reported a similar degree of apparent synergy from the addition of CCNU to the combination of CYC, ADR, CDDP, and vincristine.

In summary, we feel that we have demonstrated a role for CDDP used in combination with DAG and ADR in squamous cell lung cancer and have identified DAP as a very active combination regimen. The role of DAG needs further definition.

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Cyclophosphamide, Vincristine, Adriamycin, and DTIC (CYVADIC) Combination Chemotherapy for the Treatment of Advanced Sarcomas [1,2]

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SUMMARY

One hundred and forty adult patients with advanced sarcomas (125 soft tissue and 15 bone) were treated with a combination chemotherapy regimen consisting of cyclophosphamide, vincristine, Adriamycin, and DTIC (CYVADIC). There were 21 (15%) complete and 45 (32%) partial responders, with an overall response rate of 47%. The response rate was 50% (17% complete) for patients with soft tissue sarcomas compared with 20% (none complete) for patients with bone sarcomas. The median duration of response was 9.5 months (range, 1–40+ months) for complete responders and 7 months (range, 1–31 months) for partial responders. The median time to achieve response was 9 weeks and the median number of courses of therapy to response was three. The median survival time was 16 months for responders compared with 7 months for nonresponders ($P = 0.001$). The most responsive tumor types were neurofibrosarcoma, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma, and angiosarcoma. Pulmonary and soft tissue metastases were more responsive than bone and liver metastases. CYVADIC toxicity was predominantly limited to myelosuppression, vomiting, fever of unknown origin, and neuropathy. CYVADIC is an effective combination chemotherapy regimen in the treatment of advanced sarcomas.

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[4]We would like to express great appreciation to the late Dr. Jeffrey A. Gottlieb, who actually designed the study.

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Considerable advances have been made in the last decade in the treatment of adult advanced soft tissue and bony sarcomas. Before the introduction of Adriamycin into clinical practice, patients were usually treated with vincristine, cyclophosphamide and/or actinomycin D, either as single agents or in combination. Response rates for this type of therapy were usually < 20% in adults (1). With Adriamycin given alone, responses were seen in 27%–40% of patients (2–4). Early studies of DTIC indicated activity in metastatic sarcomas, and a 17% response rate was noted in 53 patients (5). By combining Adriamycin and DTIC, an overall response rate of 42%, with a complete remission rate of 11%, was obtained in 218 evaluable patients (4).

Sequential additions of vincristine and cyclophosphamide were then made to the Adriamycin-DTIC (A-DIC) regimen (4). The addition of vincristine (VADIC) did not improve response rates or survival (4). Cyclophosphamide, known to be active in childhood sarcomas, was then added resulting in the CYVADIC combination chemo-

therapy regimen, which was initiated in February 1973. This report is a final analysis of the 140 evaluable patients with advanced soft tissue and bony sarcomas who were treated with this regimen.

MATERIALS AND METHODS

All patients with a biopsy-confirmed diagnosis of soft tissue or bony sarcomas were eligible for this study. Only those patients who received protocol therapy and in whom adequate data were available were considered evaluable. This study was conducted as a pilot investigation at the M. D. Anderson Hospital and Tumor Institute, but ten other institutions in the Southwest Oncology Group (SWOG) collaborated and contributed approximately 40% of the evaluable patients. In order to be eligible for the study, all patients were required to have an absolute granulocyte count of $\geq 1000/mm^3$ and a platelet count of $\geq 100,000/mm^3$ at the time of initial therapy. Patients were required to have normal renal function (defined as a serum creatinine of < 2 mg/dl or creatinine clearance of > 60 ml/minute), adequate liver function (with a bilirubin of < 2 mg/dl), and no evidence of biliary obstruction. Informed consent was obtained from all patients in accordance with individual institutional policies.

All patients entering in this study with adequate bone marrow reserve received the following chemotherapeutic regimen: 50 mg/m^2 iv on Day 1 only of Adriamycin; 500 mg/m^2 iv on Day 1 only of cyclophosphamide; 250 mg/m^2/day $\times$ 5 days of DTIC; and 1.5 mg/m^2 iv (upper limit of dose = 2 mg) on Days 1 and 5 of vincristine. For patients considered to have inadequate bone marrow reserve secondary to extensive previous chemotherapy and/or radiotherapy, the same schedule was used, except that the dose of Adriamycin was 40 mg/m^2 on Day 1, the dose of cyclophosphamide was 400 mg/m^2 on Day 1, and the dose of DTIC was 200 mg/m^2/day $\times$ 5 days. Each cycle of therapy was repeated every 3 weeks if patients had recovered from toxicity. The dose in subsequent courses was modified by the degree of myelosuppression encountered during the previous course. If a patient's absolute granulocyte count had not decreased to $< 1000/mm^3$ and his platelet count had not decreased to $< 100,000/mm^3$ during the previous course, the dose in the next course was escalated by 10 mg/m^2 for Adriamycin, by 100 mg/m^2 for cyclophosphamide, and by 50 mg/m^2/day $\times$ 5 for DTIC. Doses were decreased by similar amounts for patients whose absolute granulocyte counts decreased to $500/mm^3$ or whose platelet counts decreased to $< 50,000/mm^3$, while the dose level remained the same as that used in the previous course for patients whose lowest granulocyte count fell between 500 and $1000/mm^3$ and whose lowest platelet count fell between 50,000 and $100,000/mm^3$. If the absolute granulocyte count was still $< 1000/mm^3$ and/or the platelet count was still $< 50,000/mm^3$ on Day 22 of a course of therapy, the next course of therapy was delayed until the blood cell counts had increased to these levels. Patients in the study continued to receive all four drugs every 3 weeks until definite disease progression occurred or substantial toxicity precluded further treatment. The total dose of Adriamycin was limited to 550 mg/m^2, and when this dose level had been reached, patients continued to receive DTIC, vincristine, and cyclophosphamide. All patients were followed with weekly cbcs, together with liver and renal function tests; ECGs and appropriate radiologic studies were repeated every 3–6 weeks. Tumor measurements were recorded every 3 weeks.

A complete remission (CR) was defined as the disappearance of all disease and symptoms for at least 4 weeks. A partial remission (PR) was defined as a $\geq 50\%$ reduction in the product of the longest perpendicular diameters of measurable lesions with no new lesions appearing. Stable disease was defined as a response less than a PR, or a steady state not qualifying for progressive disease of at least 8 weeks' duration. Progressive disease was defined as at least a 25% increase in the size of any measured lesion or the appearance of new lesions.

The survival calculations were made from the day of initiation of chemotherapy to the day of death and the response duration was dated from the day of maximal response to the day of any disease progression. The Kaplan and Meier method was used to calculate and plot remission and survival curves, and a generalized Wilcoxon test with a one-tailed analysis was used to test differences between remission and survival curves (6,7).

RESULTS

A total of 159 patients were treated with this regimen. Among these 159 patients, 19 were considered unevaluable because of early death (seven patients), inadequate trial (five, including two who refused further treatment), loss to followup (five), major protocol violation (one), and no measurable disease (one). Of the 140 evaluable patients, there were 70 males and 70 females ranging in age from 15 to 78 years (median, 55 years). There were 21 (15%) CRs and 45 (32%) PRs, with an overall response rate of 47% (66 of 140 patients). Forty-four patients (31%) had stable disease while 20 (22%) had disease progression. In a preliminary analysis of the first 136 evaluable patients entered in this study (4), the reported overall response rate was 55%, with 19 CRs and 56 PRs. In this final analysis, four additional evaluable patients were entered in the study. Upon careful re-evaluation and with further followup information, nine patients who were previously classified as partial responders were considered to have achieved $< 50\%$ tumor regression and have therefore been reclassified as having stable disease in this analysis. Furthermore, there were two patients among the 21 complete responders who were in partial remission at the time of the preliminary analysis.

The median duration of response was 9.5 months

(range, 1–40+ months) for CRs and 7 months (range, 1–31 months) for PRs. The median time to achieve response was 9 weeks (range, 3–32 weeks) and the median number of courses of therapy to maximal response was three (range, one to eight courses). Response according to the various histologic subtypes is shown in table 1.

Nineteen patients had previously received chemotherapy including vincristine, actinomycin D, cyclophosphamide, and methotrexate either alone or in combination. The response rate was 26% (five of 19 patients) in the previously treated patients compared with the response rate of 50% (61 of 121 patients) in those who had not received prior chemotherapy ($P > 0.05, < 1.0$). The response rate was 27% (four of 15 patients) for those patients who had previously received cyclophosphamide and 50% (62 of 125 patients) for those who had not previously received cyclophosphamide. Seven of the 19 patients (37%) who had received prior therapy had a dose reduction with the first or subsequent courses, while 48 of 121 (40%) previously untreated patients had a dose reduction.

Table 2 shows the response rate by site of metastases. The major sites of involvement were soft tissue, lung, bone, and liver. The response rates were lowest if the major site of metastasis was bone or liver. Of the 59% of patients who recurred in soft tissue, 76% represented local recurrences and 24% represented distant metastases.

The time to progression as determined from the start of chemotherapy to the date of first evidence of disease progression is shown in figure 1. The median time to progression was 12.5 months for complete responders compared with 10 months for partial responders ($P = 0.02$), 5 months for patients with stable disease ($P = 0.001$), and 2 months for patients with progressive disease ($P = 0.001$).

The duration of survival from the start of therapy is shown in figure 2. The median duration of survival was

TABLE 1.—Response by tumor type

Tumor type	No. of patients	No. of CRs/PRs	Response rate (%)
Soft tissue			
Angiosarcoma	5	0/3	60
Fibrosarcoma	21	4/6	48
Leiomyosarcoma	31	4/14	58
Liposarcoma	12	1/4	42
Mesothelioma	6	1/1	33
Neurofibrosarcoma	13	4/5	69
Rhabdomyosarcoma	13	5/2	54
Synovial sarcoma	2	0/1	50
Undifferentiated sarcoma	9	2/3	56
Miscellaneous sarcoma	13	0/3	23
Totals	125	21/42	50
Bone			
Chondrosarcoma	4	0/0	0
Ewing's sarcoma	2	0/1	50
Osteogenic sarcoma	9	0/2	22
Totals	15	0/3	20
Overall totals	140	21/45	47

TABLE 2.—Response by site of metastases

	No. of patients			
	Soft tissue sarcoma	Bone sarcoma	Total	% of patients responding
Soft tissue	77	6	83	45
Lung	66	12	78	58
Pleura	7	1	8	50
Bone	10	5	15	33
Liver	21	0	21	38

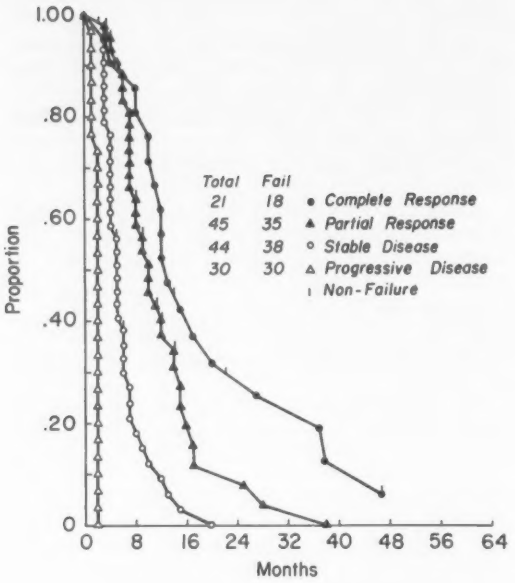

FIGURE 1.—CYVADIC: time to progression.

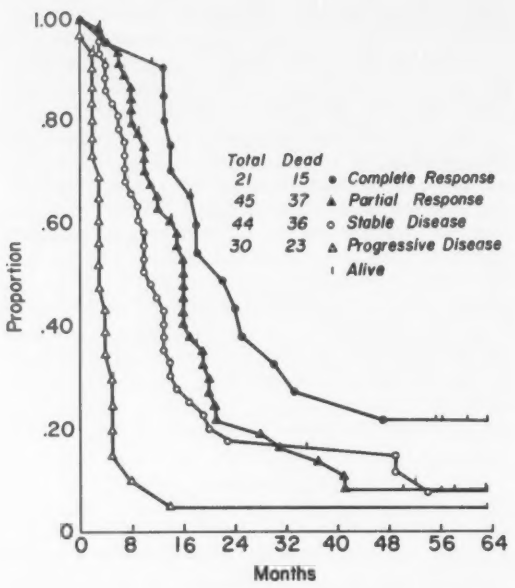

FIGURE 2.—CYVADIC: duration of survival.

21 months (range, 4–63+ months) for complete responders, 16 months (range, 3–63+ months) for partial responders, 10 months (range, 3–63+ months) for patients with stable disease, and 3.0 months (range, 2–63+ months) for patients with progressive disease. The duration of survival for patients achieving CR was significantly longer than that for patients achieving PR ($P = 0.01$), which was significantly longer than that for patients with stable disease ($P = 0.02$). The P value is 0.001 for stable disease compared to progressive disease. The median survival time was 16 months for all responders versus 7 months for all nonresponders ($P = 0.001$).

Side effects of this regimen are shown in table 3. Nausea and vomiting occurred in the majority of patients and was particularly severe on Day 1. Almost one fourth of the patients had fever of unknown origin associated with neutropenia. Thirteen patients developed pneumonia and septicemia, and four patients with neutropenia in this group died. Three other patients developed Adriamycin cardiomyopathy, one of whom died of resulting congestive cardiac failure. Fifty-five patients had a dose reduction in an attempt to maintain a 3-week schedule. The lack of myelosuppression allowed a dose escalation to be made for eight patients.

Table 4 shows the degree of myelosuppression produced by this combined treatment. Maximum myelosuppression occurred in most patients on Day 14 and there was rapid recovery by Day 21. There appeared to be some cumulative toxicity as shown by a slightly greater decrease in neutrophil counts with subsequent treatment courses.

DISCUSSION

Combination chemotherapy with the CYVADIC regimen in adult soft tissue and bony sarcomas has produced

TABLE 3.—Nonhematologic toxicity

Toxic effect	% of patients
Nausea and vomiting	85
Fever of unknown origin with neutropenia	22
Vincristine neuropathy	13
Pneumonia and septicemia	9
Severe stomatitis	4
Urinary tract infection	2
Adriamycin cardiomyopathy	2
General malaise and weight loss	2
Paralytic ileus	2
Constipation	2
Diarrhea	2
Gastrointestinal hemorrhage	1
Hemorrhagic cystitis	1
Skin rash	1

TABLE 4.—Hematologic toxicity

Toxic effect	Course 1	Course 2	Course 3	Course 6
Wbc count (cells × 10^3/mm^3)				
Median (Day)	2.2(14)	2.4(14)	2.3(14)	1.7(14)
Range (Days)	0.5–6.0(8–21)	0.4–7.7(5–22)	0.1–11.3(8–21)	0.5–3.9(8–21)
Absolute granulocyte count (cells × 10^3/mm^3)				
Median (Day)	1.1(14)	1.5(14)	1.0(14)	0.7(14)
Range (Days)	0–5.7(8–21)	0–4.8(5–22)	0–9.9(14)	0.2–2.7(8–21)
Platelet count (cells × 10^3/mm^3)				
Median (Day)	198(13)	200(14)	180(15)	185(14)
Range (Days)	32–544(8–22)	8–515(5–22)	12–652(8–22)	14–441(5–28)
% of dose given				
Median	100	100	100	100
Range	80–100	60–120	60–120	60–120

an overall response rate of 47%, with 15% of patients achieving complete remission. The response rate was 50% (17% complete) for soft tissue sarcomas compared with 20% (none complete) for bony sarcomas. All types of sarcomas responded to the regimen except chondrosarcoma. The most responsive tumor types were neurofibrosarcoma, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma, and angiosarcoma. Patients who had received prior chemotherapy had a poorer response than those who had not. Pulmonary and soft tissue metastases were more responsive than bone and liver metastases.

In a study of Adriamycin and DTIC conducted by the SWOG, an overall response rate of 42%, with 11% complete remission, was observed in 218 evaluable patients (4). Thirty-three percent of the patients had stable disease while 25% had disease progression. Like the CYVADIC regimen, responses were seen in all tumor types except chondrosarcoma. The median duration of survival was 15 months for all responders. Thus, the CYVADIC regimen appears to be only slightly more effective than the Adriamycin-DTIC combination, both in terms of response rate and duration of survival. One reason for the small difference in response may be that the dose of Adriamycin in the CYVADIC regimen had to be reduced to allow for the inclusion of cyclophosphamide.

The response rate observed in this study is comparable to that obtained by the SWOG comparing the CYVADIC regimen with a similar regimen in which actinomycin D was substituted for DTIC (CYVADACT) (8). The overall response rate was 52% (14% complete) for CYVADIC in the first 221 evaluable patients and 40% (12% complete) for CYVADACT in 224 evaluable patients ($P = 0.02$). A superior response rate for the CYVADIC regimen was observed in the treatment of fibrosarcoma, leiomyosarcoma, mesothelioma, and undifferentiated sarcoma. The median survival was 19 months for CYVADIC responders versus 12 months for CYVADACT patients ($P = 0.006$). Survival with the CYVADIC regimen was not significantly superior to the 15-month median survival duration observed with previous studies with the Adriamycin-DTIC combination with or without vincristine ($P = 0.24$).

Other investigators have used a number of agents including Adriamycin, DTIC, methotrexate, vincristine, cyclophosphamide, actinomycin D, and methyl-CCNU in various combinations to treat adult sarcomas (9). The response rates were low when Adriamycin was not included, and most of the combinations with Adriamycin but without DTIC did not appear to be superior to Adriamycin alone. Nevertheless, a recent comparison of three regimens by Subramanian and Wiltshaw (10) indicated that the response rate for methotrexate alone was comparable with that of a five-drug regimen (methotrexate, cyclophosphamide, vincristine, 5-fluorouracil, and actinomycin D) and that of a three-drug combination (methotrexate, Adriamycin, and DTIC). Responses were obtained in 41 patients (37%; 5% complete) treated with methotrexate alone, in 12 patients (67%; 25% complete) treated with the five-drug regimen, and in 22 patients (41%; 15% complete) treated with the three-drug regimen.

CYVADIC appears to be the most effective combination chemotherapy regimen for the treatment of adult metastatic sarcomas, particularly of the soft tissues. It is presently our treatment of choice for front-line therapy for metastatic soft tissue sarcomas. However, in view of significant toxicity associated with its use, and since the addition of vincristine to the Adriamycin-DTIC combination did not improve response rate or survival, we are currently investigating the relative contribution of cyclophosphamide or actinomycin D to Adriamycin and DTIC. Other studies are also in progress to investigate

new agents, to explore different schedules or routes of administration of established agents (eg, continuous infusion and intra-arterial therapy), and to treat patients in the protected environment employing intensive chemotherapy with or without autologous bone marrow transplantation. Treatment should be as aggressive as possible since occasional patients with advanced soft tissue sarcomas may be cured with chemotherapy with or without surgery (11).

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A Sensitive and Specific Assay for Pentamethylmelamine in Plasma: Applicability to Clinical Studies [1]

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SUMMARY

A sensitive and specific procedure for measuring plasma levels of pentamethylmelamine (PMM), a chemotherapeutic agent currently undergoing phase I trials, has been developed. PMM was isolated (recovery > 95%) from 3-ml plasma samples by cation exchange chromatography followed by solvent extraction, and was quantitated by selected ion monitoring with a gas chromatograph/mass spectrometer. Deuterium-labeled PMM was synthesized and used as an internal standard; standard curves were prepared by plotting m/e 196/199 ratios versus concentrations, and were linear in the range of 0.05–50.0 μM. The coefficient of variation for repeated measurements was < 1% at all concentrations studied. To test the applicability of this method to clinical studies, plasma clearances of PMM were measured in two studies in each of three cancer patients receiving 80-mg/m^2 doses of PMM as 1-hour iv infusions. Peak plasma concentrations, observed immediately after the end of the infusions, averaged 4.1 μM (SE = 0.6 μM). Plasma elimination was found to be biphasic; a short initial half-life (approximately 27 minutes) was followed by a longer elimination half-life of 133 minutes (SE = 35 minutes). Measurements in two patients indicated that < 0.1% of the administered PMM was excreted unchanged in the urine in 24 hours, suggesting that hepatic metabolism of PMM may be a major contributor to plasma clearance of the drug in man.

[Cancer Treat Rep 64:99–104, 1980]

Phase I clinical trials of pentamethylmelamine (PMM) have recently been initiated at several institutions. PMM is a metabolite of hexamethylmelamine (HMM) (1), a drug that has been used clinically in the treatment of lymphomas and tumors of the ovary, cervix, breast, and lung (2). While the use of HMM has yielded promising results in patients with these tumors, the poor solubility of the compound in suitable vehicles has prevented its iv use. In virtually all human studies, HMM has been administered orally, and treatment failures may have resulted from dose-limiting gastrointestinal toxic effects (2) or inadequate or unpredictable absorption. The aqueous solubility of PMM is 24 times that of HMM (3,4); this property has led to its current investigational use in iv infusions in cancer patients. In animal test systems, PMM has shown a spectrum of antitumor activity similar to that of HMM, and it is approximately equipotent with HMM (3–5). Demethylated analogs of HMM, which are metabolites of HMM (1) and possible metabolites of PMM, have less antitumor activity.

Suitable analytic methods for measuring plasma PMM concentrations would be desirable for the optimization of the therapeutic index of the drug, and would facilitate the development of correlations between therapeutic responses, dose-limiting toxic effects, and pharmacokinetics in the protocols under study. We report here a sensitive and specific assay for plasma PMM that utilizes gas

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[3]We wish to thank Drs. Daniel C. Ihde and Robert C. Young for providing the patient samples used in this study.

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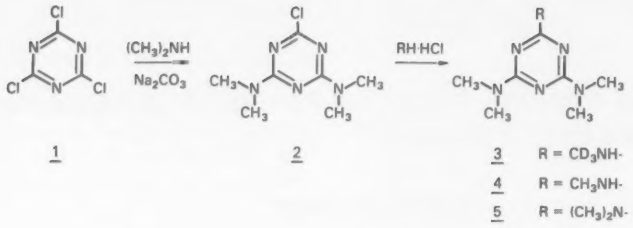

FIGURE 1.—Schematic representation of the synthesis of deuterium-labeled PMM (*3*) and the chemical structures of PMM (*4*) and HMM (*5*).

chromatography/mass spectrometry, and we describe the synthesis of deuterium-labeled PMM which is used as an internal standard in the assay. Preliminary pharmacokinetic studies of PMM in three patients are presented to illustrate the application of the assay in the clinical setting and to suggest some important considerations for more definitive pharmacokinetic and therapeutic studies.

MATERIALS AND METHODS

Chemicals

Pentamethylmelamine hydrochloride (*4*; *see* fig 1) and pentamethylmelamine ([^{14}C]-methylamine) hydrochloride (15.8 millicuries/mmol) were supplied by Dr. Robert R. Engle, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Cyanuric chloride (*1*), [D_3]-methylamine hydrochloride (98+ atom % D), and dimethylamine (40% aqueous solution) were purchased from Aldrich Chemical Co, Milwaukee, WI. Biorex 70 (200–400 mesh, sodium form), a carboxylic cation exchange resin, was purchased from Bio-Rad Laboratories, Richmond, CA. Preparative thin-layer chromatography plates (1000μ silica gel GF) were purchased from Analtech, Inc, Newark, DE. All other chemicals and solvents were reagent grade.

Synthesis of ([D_3]-Methylamine)-4,6-bis(dimethylamine)-S-triazine (D_3-PMM)

The internal standard for this assay, deuterium-labeled PMM, was synthesized as shown in figure 1 using an adaptation of the procedure used for the synthesis of [^{14}C]-PMM.[4] A solution of 4.5 g (24.5 mmols) of cyanuric chloride (*1*) and 30 ml of acetone was magnetically stirred and cooled in an ice bath. To this was added 2.7 g (25.5 mmols) of Na_2CO_3, 8.2 ml (72.9 mmols) of 40% aqueous dimethylamine, and 25 ml of H_2O. Stirring was continued for 5 hours, after which the reaction mixture was concentrated in vacuo, and the white solid was filtered, washed with three 30-ml portions of cold H_2O, and dried to yield 3.6 g (73%) of chloride (*2*). A magnetically stirred solution containing 500 mg (7.1 mmols) of [D_3]-methylamine hydrochloride, 1.5 g (8.2 mmols) of chloride (*2*), 7 ml of H_2O, and 9 ml of 1 N NaOH was heated in a 15 × 250-mm sealed tube at 100°C for 12 hours. The contents of the tube were shaken with 70 ml of $CHCl_3$ and 70 ml of H_2O; the organic solution was separated and evaporated. This crude product (1.6 g) was purified by preparative thin-layer chromatography (20 × 20-cm silica gel plates, 1000μ; 30% EtOAc-hexane; R_f = 0.4) yielding 0.73 g (52%) of D_3-PMM as a white solid.

The identity of compounds *2* and *3* was confirmed by mass spectral analysis.

Patients

Three patients who had experienced progressive tumor growth during conventional treatment regimens were selected for study. Patient A was a 55-year-old woman with primary breast carcinoma and metastatic lesions in bone, liver, and central nervous system. Patient B was a 68-year-old man who had synovial cell sarcoma (left arm) with hepatic metastases. Patient C was a 69-year-old man with nasopharyngeal carcinoma and pulmonary metastases. Patients A and C were hospitalized at the Clinical Center, National Institutes of Health, through the Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, and patient B was at the Veterans' Administration Hospital, Washington, DC. Patient A had elevated liver function parameters which increased further during this study: SGOT increased from 39 to 129 (normal, 8–31), SGPT increased from 82 to 233 (normal, 3–44), alkaline phosphatase increased from 353 to 640 (normal, 21–117), and lactate dehydrogenase increased from 353 to 869 (normal, 129–381) (all values are expressed in IU per liter). Liver

[4]Leffer MA, SRI International. The synthetic procedure for [^{14}C]-PMM was developed under contract N01-CM-63776 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and this procedure was supplied by Dr. R. R. Engle, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.

function was normal and remained constant for patients B and C. Kidney function, as measured by blood urea nitrogen and serum creatinine, was normal during this study in all patients.

Sample Collection and Assay Procedures

PMM (80 mg/m^2) was administered in 250 ml of 5% dextrose solution as a 1-hour iv infusion using an iMed Volumetric Infusion Pump (model 922, iMed Corp, San Diego, CA). At timed intervals after the infusion, blood samples were drawn through a heparin lock placed at a site distant from the infusion site. Blood was collected into heparinized Vacutainer tubes (7 ml, Fisher Scientific Co, Pittsburgh, PA). After centrifugation at 1000 × *g* for 20 minutes, the plasma was separated and stored at –78°C prior to analysis. Aliquots of 24-hour urine samples collected from patient A (trial 1) and patient C (trial 1) were also stored at –78°C.

The procedure for extraction of PMM from plasma is outlined in figure 2. The thawed plasma samples were recentrifuged at 1000 × *g* for 20 minutes to remove microparticulate matter. Three ml of plasma mixed with 1 ml of 1 N HCl and 1 ml of internal standard solution (2-μg/ml aqueous solution of the hydrochloride of *3*) was applied to a cation exchange column (5 × 0.8-cm Biorex 70, hydrogen form). The column was washed with 5 ml of 1 N HCl, 30 ml of water, and 11 ml of 1 N NaOH. The final 6 ml of NaOH solution was collected in a 15-ml ground-glass stoppered centrifuge tube and shaken with 5 ml of diethyl ether for 30 seconds. The ether solution

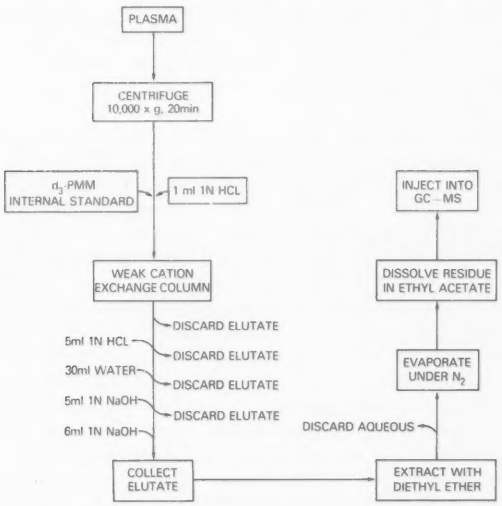

FIGURE 2.—Schematic diagram of the analytic procedure for quantitating PMM in plasma.

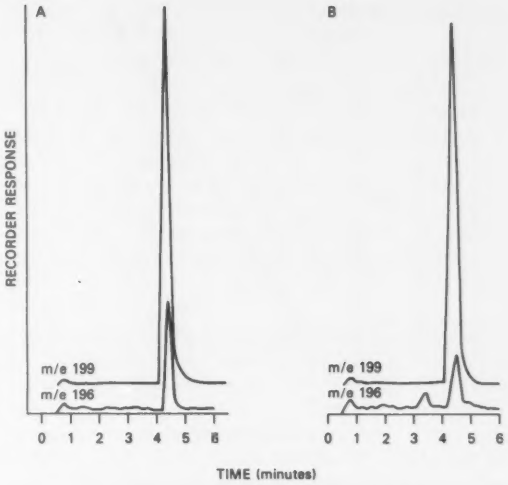

FIGURE 3.—Selected ion-current profiles of extracts of (A) a plasma standard containing 1 × 10^{-7} M PMM, and (B) plasma from a patient receiving 80 mg/m^2 of PMM as a 1-hr infusion (sample drawn 780 mins after cessation of infusion). Profile B corresponds to a concentration of 4.9 × 10^{-8} M PMM (the m/e 199 signal has been divided by a factor of 10).

was transferred to a 5-ml Reacti-Vial (Pierce Chemical Co, Rockford, IL) and evaporated under a stream of nitrogen.

Samples were analyzed by selected ion monitoring using a Varian MAT-44 Gas Chromatograph-Quadrapole Mass Spectrometer (Varian-MAT, Florham Park, NJ) in the electron impact mode. The gas chromatograph was fitted with a 2-mm × 2-m glass column packed with 3% OV-17 liquid phase on a solid support of Chromosorb W/HP (Varian Instrument Division, Sunnyvale, CA) which was silylated with Col-Treet (Regis Chemical Co, Rockford, IL) prior to each set of analyses. The temperature of the injection port, separator, and transfer line was 210°C, and that of the column oven was 160°C. Helium carrier gas was maintained at a flow rate of 30 ml/minute. The ionizing voltage was 70 eV. The sample residue was dissolved in 25 μl of ethyl acetate and 5 μl was injected into the gas chromatograph. Since the base peak (most intense ion) of both compounds was the molecular ion, masses 196 (PMM) and 199 (D_3-PMM) were monitored (fig 3) and the area ratio of m/e 196/199 was used for quantitation. Standards prepared from pooled human plasma were analyzed along with the patient samples. Standard curves, constructed by plotting the area ratio of m/e 196/199 versus concentration (fig 4), were used to determine the concentration of PMM in the patient samples.

Urine was assayed for PMM by the same procedure

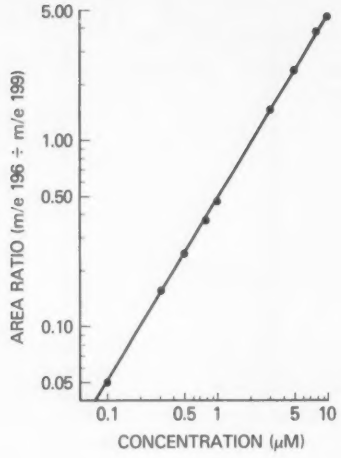

FIGURE 4.—Standard curve for PMM extracted from plasma and analyzed as described in the Materials and Methods section.

used for plasma, except that (*a*) 10-ml samples were used, (*b*) the drug was extracted from the column eluate with hexane, and (*c*) standards were prepared in urine.

Pharmacokinetic Analyses

Plasma PMM concentrations were fit to a two-compartment model (6) using the MLAB Curve Fitting Program (7) implemented on a PDP-10 Control Data Corp computer. Parameters from each analysis were used to calculate best-fit plasma concentration versus time curves (fig 5) and terminal-phase half-life values (table 1).

RESULTS AND DISCUSSION

A clinically useful analytic procedure to determine plasma drug levels must have sufficient sensitivity to detect the drug several half-lives after administration and must also have sufficient specificity to avoid interference by metabolites and other compounds commonly found in the blood of patients being studied. As with HMM (8), a short plasma half-life was expected for PMM, necessitating a sensitive analytic procedure with an appropriate internal standard. Furthermore, the possibility of high plasma levels of structurally similar metabolites (formed by successive demethylation) required an analytic technique of high specificity. We therefore chose to develop an assay based on gas chromatography/mass spectrometry because of its demonstrated sensitivity and

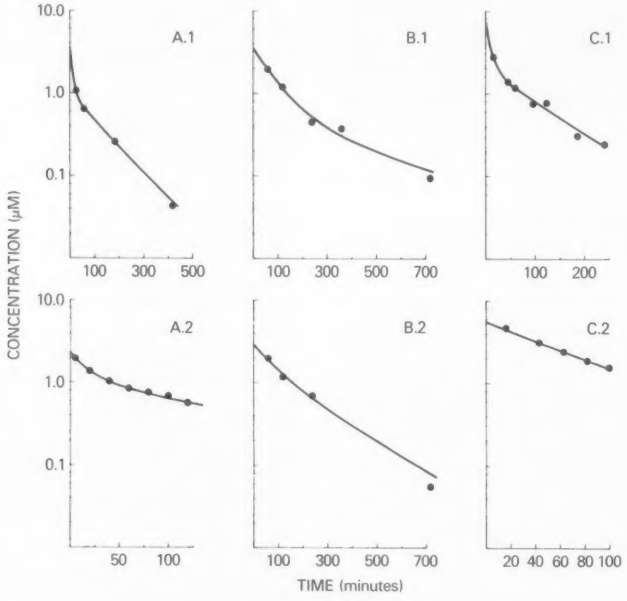

FIGURE 5.—Plasma concentrations in 3 patients (A, B, and C) receiving PMM (80 mg/m²) as a 1-hr iv infusion determined by (*a*) analysis as described in the text (●), and (*b*) computer fit by a 2-compartment pharmacokinetic analysis of the data (———). Note the differing time scales between trials.

TABLE 1.—Plasma elimination half-lives and peak levels in patients receiving 1-hr iv infusions (80 mg/m²) of PMM

Patient	Trial	Half-life (mins)	Peak level (μM)
A	1	97	3.7
	2	115	2.3*
B	1	290	3.4*
	2	167	3.0
C	1	77	6.4
	2	52	5.6*
Mean + SE		133 ± 35	4.1 ± 0.6

*Estimated from the pharmacokinetic model when no sample was obtained immediately after the end of the infusion.

specificity and the relative ease of preparing an appropriate internal standard.

To correct for variability in extraction efficiencies, recoveries, and manipulative losses, D_3-PMM was synthesized for use as an internal standard (fig 1). A simple extraction from plasma provided inadequate preparation for quantitation of PMM by gas chromatography/mass spectrometry. Continued degradation of chromatographic characteristics, nonreproducibility following multiple injections of the same extract, and nonlinear standard curves prepared from plasma standards indicated that a more effective sample cleanup was necessary for this assay. This was accomplished as shown in figure 2. After adding internal standard to the plasma sample, PMM and D_3-PMM were separated from potentially interfering neutral and acidic substances by passing the sample through a weak cation exchange resin. The compounds were then eluted from the column with aqueous sodium hydroxide and further purified by solvent extraction from the eluate. Groups of 30–40 samples can be conveniently processed at one time, the total cleanup procedure requiring about 5 hours. Recovery of PMM from a 100 μM standard solution containing ^{14}C-labeled PMM was 96% from the ion exchange column and 99% from the extraction.

Three extractions of the same sample gave coefficients of variation of 0.9%, 0.7%, and 1.0% for 10.0, 1.0, and 0.1 μM standards respectively. The coefficient of variation for multiple injections of the same sample extract was 0.3% for a 10 μM standard (n = 3) and 2.5% for a 0.1 μM standard (n = 3). Because of this excellent reproducibility, only one extract was prepared from each patient sample and each extract was injected twice into the gas chromatograph/mass spectrometer. The relative sample purity also allowed rapid analysis, as the gas chromatographic retention time for PMM was 4.4 minutes. Typical ion-current profiles of extracts from a 1×10^{-7} M PMM plasma standard (A) and a plasma sample from a patient after administration of PMM (as described in the Materials and Methods section) which contained 4.9×10^{-8} M PMM (B) are shown in figure 3.

Figure 4 illustrates the excellent linearity ($r^2 = 0.997$) of a typical standard curve. Using 3 ml of plasma, the lower limit for satisfactory quantitation (coefficient of variation for multiple injections of the same sample = < 5%) was approximately 50 nM. Lower concentrations could likely be measured by using larger plasma samples and/or injecting more of the extract into the gas chromatograph/mass spectrometer, although this was not necessary in the present study. We have found the standard curve to be linear over at least a 1000-fold concentration range (0.05–50 μM).

The assay procedure was applied to preliminary studies of PMM during initial clinical trials of this agent. PMM (80 mg/m²) was administered at weekly intervals to three cancer patients. Figure 5 shows plasma concentration versus time curves obtained during the first two infusions for patients A and B and the first and third infusions for patient C. The points represent the plasma concentrations of PMM measured at different times, and the solid lines represent the computer fit obtained using a two-compartment pharmacokinetic model (6). In order to improve the pharmacokinetic analysis of the data, sampling times were altered as the kinetics of the drug became known. For instance, since the distribution phase of the drug seemed to be relatively rapid (half-life = 6.7 minutes for patient A, trial 1), samples were drawn at earlier times for trial 2. Table 1 lists the elimination half-lives and the observed peak concentrations.

The plasma curve for patient C (trial 2) appears to be monoexponential. Since the calculated half-life was shorter than for trial 1, and also because in trial 2 the first sample was not drawn until 16 minutes after cessation of the infusion, the distribution phase was likely missed altogether in this particular trial.

Analysis of pretreatment plasma samples demonstrated that none of the following concurrent medications or their metabolites gave peaks which interfered with this PMM assay: dexamethazone, phenytoin, perchlorperazine, codeine, phenyltoloxamine acetate, and Tylenol.

Analysis of urine demonstrated that < 0.1% of the dose was excreted by the kidney as PMM in 24 hours by patient A (trial 1) and patient C (trial 1).

This assay can also be used to simultaneously quantitate HMM and PMM in plasma. A preliminary study in one patient showed plasma levels of 1.7 and 0.6 μM of HMM and PMM 1 hour after a single oral dose of 140 mg/m² of HMM, demonstrating that PMM is a metabolite of HMM in man.

In conclusion, the assay for plasma PMM reported here possesses the sensitivity and selectivity required for studies of PMM pharmacologic action in man. The dose

studied in all three of the patients was the lowest dose planned in protocols currently under investigation. Future studies undoubtedly will employ larger PMM doses, and plasma PMM concentrations are expected to be correspondingly higher. In view of the rather short half-lives observed in these studies, in future investigations plasma samples should be drawn at times soon after drug administration in order that accurate determinations of the α- and β-phases of plasma PMM elimination kinetics can be made. Because suspected metabolites (demethylated products) of PMM have less antitumor activity (3–5), and it appears likely that PMM is metabolized extensively in man, pharmacokinetic studies centering on the parent drug should be helpful for the optimization of therapeutic regimens in the clinical setting. However, this technique should be readily adaptable for monitoring certain suspected demethylated metabolites of PMM, should this prove to be necessary.

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Amygdalin (Laetrile): Effect on Clonogenic Cells From Human Myeloid Leukemia Cell Lines and Normal Human Marrow[1,2]

H. Phillip Koeffler,* Leslie Lowe, and David W. Golde[3,4]

SUMMARY

The effect of amygdalin on human acute myelogenous leukemia cells and normal bone marrow granulocyte-monocyte precursors was studied in vitro using soft-gel culture. A pharmaceutical and a clinical source of amygdalin were tested and β-glucosidase was added to selected cultures to promote the hydrolysis of amygdalin. Acute myelogenous leukemia cells were obtained from two human cell lines designated KG-1 and HL-60. A 50% inhibition of colony formation by both normal and leukemic cells was observed at an amygdalin concentration of 3.5 mg/ml using both drug sources. We found the colony-forming cells from the leukemic cell lines and normal marrow to be relatively resistant to amygdalin and its metabolites in vitro, and there was no selective kill of clonogenic cells from the human leukemia cell lines as compared to normal bone marrow.

[Cancer Treat Rep 64:105–109, 1980]

Amygdalin (Laetrile) contains small amounts of hydrogen cyanide (HCN). Proponents of its use state that cancer cells have abundant amounts of the enzyme β-glucosidase which catalyzes the hydrolysis of amygdalin and the subsequent release of HCN (1–3). The cancer cell is further stated to be deficient in rhodanese (thiosulfate sulfurtransferase), the enzyme catalyzing the conversion and inactivation of HCN to a thiocynate. Normal cells are purported to have low levels of the HCN-activating enzymes and to be rich in rhodanese. The theoretic basis for the use of amygdalin, therefore, is that cancer cells are preferentially killed by amygdalin and normal cells are spared.

Amygdalin is commercially produced from a variety of plants including apricot, peach, plum, and bitter almond kernels (2,4). Ernst Krebs reported that Laetrile was mandelonitrile-β-glucuronide and was obtained from amygdalin (mandelonitrile-β-D-glucoside-6-β-glucose) by an elaborate multi-step chemical synthesis (Krebs ET, and Krebs ET, Jr, US patent 2,985-664, issued in 1960; British patent 788,855, issued in 1958). Careful pharmacologic assessment, however, has shown that the material known in the United States as Laetrile is amygdalin (5).

Amygdalin was found to have no therapeutic value in various animal tumor systems (6–8). A number of uncontrolled clinical studies suggested that the drug was ineffective in treating human malignancies (1,2,9). We tested the effect of amygdalin on human leukemic cells and normal myeloid precursors in vitro. We found that amygdalin products have no preferential cytotoxicity to the colony-forming cells from human acute myelogenous leukemia (AML) cell lines as compared to normal human bone marrow granulocyte-monocyte clonogenic cells.

MATERIALS AND METHODS

Amygdalin was obtained from Sigma Chemical Co (St. Louis, MO) and was dissolved in phosphate-buffered sa-

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[3]Division of Hematology-Oncology, Department of Medicine, University of California School of Medicine, Los Angeles, and the Veterans' Administration Hospital, Sepulveda, CA.

[4]We thank James Chien-Cheng Yen for his expert technical assistance, and Drs. Steven J. Collins and Robert C. Gallo for generously providing the HL-60 cells.

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line. Ampules of amygdalin were also obtained from the Contreras Clinic (Tijuana, Baja California, Mexico). The vials were labeled Kemdalin Amigdalina, and contained 10 ml of a clear, almond-smelling liquid said to contain 3 g of Kemdalin Amigdalina (amygdalin).

β-Glucosidase catalyzes the hydrolysis of amygdalin to glucose, benzaldehyde, and HCN. β-Glycosidase (purchased from Calbiochem, San Diego, CA), 1000 IU of enzyme per mg, was incubated at a concentration of 5 μg/ml directly with both preparations of amygdalin for 15 minutes before the drugs were added to cell cultures. Initial studies demonstrated that 5 μg/ml of β-glucosidase produced optimal amygdalin toxicity against normal marrow, HL-60, and KG-1 myeloid colony-forming cells. In control experiments, β-glucosidase alone (5 μg/ml) was found to have no effect on colony formation in vitro in leukemic or normal bone marrow cultures. Amygdalin without β-glucosidase was also added to selected cultures.

Human AML cells were obtained from two cell lines, KG-1 and HL-60. The KG-1 cell line was derived from a patient with erythroleukemia evolving into AML (10). These cells have the morphologic and karyotypic characteristics of the patient's malignant cells. The KG-1 cell line has been in continuous culture for > 1½ years and retains morphologic and cytochemical properties of myeloid leukemia cells. The other human AML cell line is known as HL-60 and was derived from a patient with promyelocytic leukemia (11). These cells also retain their leukemic morphologic features and the karyotype of the fresh leukemia cells, and are able to form tumors in nude mice.

Both KG-1 and HL-60 cells formed myeloid colonies in soft-gel culture in the presence of colony-stimulating activity (CSA). The KG-1 cells have a prominent response to CSA. In the presence of optimal CSA concentrations, the cloning efficiency was 3%, which is a 32-fold increase in colony number over plates not containing CSA. The HL-60 cells had a cloning efficiency of 7.4% in plates without CSA and there was a 2.2-fold increase in colony number when optimal concentrations of CSA were added. The CSA dose-response curves for both KG-1 and HL-60 cells consistently showed maximal colony formation with 5% conditioned medium (CSA) in the plates. The CSA source was conditioned medium from a continuous culture of T lymphocytes derived from a patient with hairy-cell leukemia (12). The cells were plated at 5×10^3 (KG-1) and 2×10^3 (HL-60) leukemia cells per dish as previously described (10) in 0.3% agar containing optimal concentrations of CSA (5%). The KG-1 cells formed 173 ± 6 colonies per 5×10^3 cells and the HL-60 cells formed 148 ± 5 colonies per 2×10^3 cells when plated in control cultures containing 5% CSA.

The same lot of fetal calf serum and CSA was used for all experiments. Amygdalin and amygdalin preincubated with β-glucosidase were added directly to the culture dishes. Plates were incubated in a humidifed atmosphere of 5% CO_2 in air for 12 days and colonies containing ≥ 50 cells were enumerated with an inverted microscope.

Amygdalin effects on normal human granulocyte-macrophage progenitors were also tested. Bone marrow was aspirated from the iliac crest of six appropriately informed healthy volunteers. The nucleated cells were isolated as previously described (13), and plated with maximally stimulating concentrations of CSA (5%). After 10 days of culture, colonies containing > 50 cells were enumerated. In control plates, 124 ± 6 colonies were formed per 2×10^5 nucleated marrow cells plated.

The activity of amygdalin and amygdalin preincubated with β-glucosidase were tested by injecting the agents into the intraperitoneal cavity of Swiss-Webster mice (Simonsen, Inc, Gilroy, CA) weighing 25–40 g. Each concentration of drug was tested in three mice and the mice were observed for 14 days after injection.

RESULTS

Amygdalin from Sigma Chemical Co caused slight inhibition of leukemia and normal cell colony formation at a concentration of 500 μg/ml (fig 1). Three milligrams per milliliter of amygdalin caused approximately a 50% decrease in myeloid colony formation by cells from both leukemic cell lines and normal human marrow. At an amygdalin concentration of 9 mg/ml, only rare colonies were seen with normal and leukemic cells. The amygdalin was not preferentially inhibitory to the proliferation of cells from the AML lines as compared to normal marrow.

The Kemdalin Amigdalina from the Contreras Clinic was also tested. The ampules are purported to contain 3 g of amygdalin. The inhibition curves were similar to those seen with the commercial Sigma preparation (fig 2). Slight colony inhibition was seen at 500 μg/ml. Approximately 3.7 mg/ml of Kemdalin Amigdalina produced about 50% colony inhibition and 9 mg suppressed almost all colony formation. The toxicity of this product to the leukemic and normal myeloid colony-forming cells did not significantly differ.

Preincubation of the Kemdalin Amigdalina with β-glucosidase enhanced its cytotoxicity (table 1). The β-glucosidase treatment decreased HL-60, KG-1, and normal marrow myeloid colony formation a mean (± SEM) of 22.8 ± 3.9, 23.3 ± 3.0, and 23.2 ± 3.7 respectively. Similar enhancement of amygdalin toxicity was seen against HL-60, KG-1, and normal human bone marrow cells using the amygdalin preparations from Sigma Chemical Co. The effect of both amygdalin preparations was tested in vivo. Concentrations of ≥ 100 mg/kg of either Kemdalin Amigdalina or Sigma amygdalin preincubated with β-glucosidase (10 mg/kg) killed mice within 5 minutes of ip injection. The β-glucosidase alone appeared not to affect the

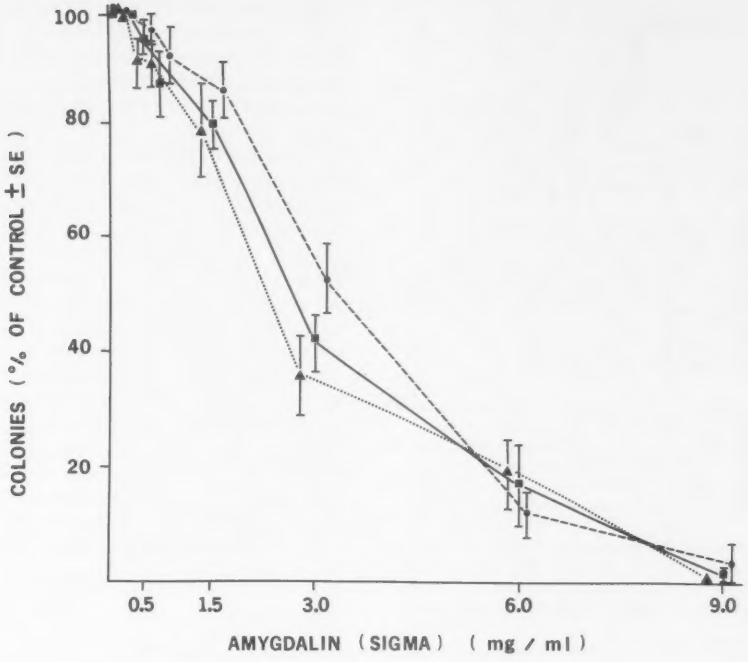

FIGURE 1.—Amygdalin (Sigma) effect on AML and normal bone marrow colony formation in vitro. ●---● = HL-60 colony-forming cells; ▲. . .▲ = KG-1 colony-forming cells; and ■——■ = human granulocyte-macrophage progenitors. Each point represents the mean ± SEM of 3–6 experiments performed in triplicate.

mice. Concentrations of ≥ 1500 mg/kg of either amygdalin product without β-glucosidase were required to kill mice within 14 days of injection.

DISCUSSION

Amygdalin has been tested in experimental animals and has not been shown to be an effective chemotherapeutic agent (6–8). Only one unconfirmed preliminary report suggests that it may have any efficacy in a murine tumor model (14). Clinical studies using the drug have been uncontrolled, but the weight of evidence suggests that the drug has no therapeutic value (1,2,9). Several clinical reports (15–18), most from outside the United States, have suggested some effect on human tumors. One in vitro study of the effect of amygdalin on macromolecular synthesis of human tumors has been reported by Levi et al (19). They showed that amygdalin at low concentrations had little effect on DNA, RNA, and protein production in tumor cells.

We found that amygdalin did not selectively kill proliferating myeloid cells from two human leukemia cell lines as compared to normal cells. Both amygdalin products appeared to have nearly equal cytotoxicity. A recent analysis of the amygdalin content of Kemdalin Amigdalina done by Cyto Pharma of Mexico has shown that it frequently contains 50% of the reported amount of the drug (20). The investigators also found that some of the ampules contained endotoxin, fungi, and isopropyl alcohol. Analysis of amygdalin samples in a Canadian study in 1965 (19) found amygdalin concentrations of 87%–98%.

The mechanism of amygdalin cytotoxicity is unknown and was not studied in our experiments. The β-glucosidase was found to enhance amygdalin's toxicity in vitro and in vivo, suggesting that HCN had a role in inhibiting colony formation. It is possible that β-glucosidase hydrolyzed amygdalin to mandelonitrile which is cleaved to yield glucose, benzaldehyde, and HCN (2,3,21). HCN binds tightly to the ferric ion of the cytochrome oxidase which inactivates this key respiratory enzyme (22,23).

We noted slight cytotoxicity of amygdalin at a concentration of 500 μg/ml in vitro. If the drug had been completely hydrolyzed by the β-glucosidase, 30 μg/ml of HCN would have been released. Fatal doses of HCN for hu-

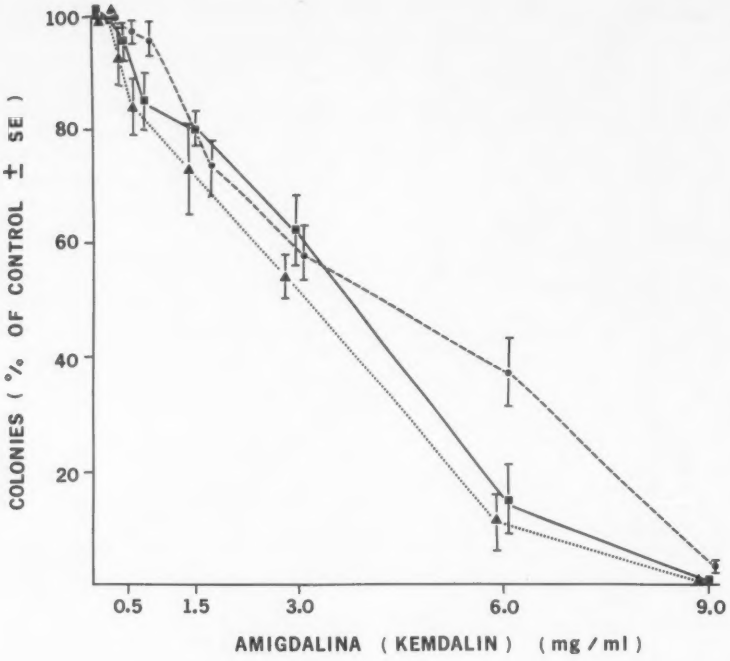

FIGURE 2.—Kemdalin Amigdalina effect on AML and normal bone marrow colony formation in vitro. ●---● = HL-60 colony-forming cells; ▲. . .▲ = KG-1 colony-forming cells; and ■——■ = human granulocyte-macrophage progenitors. Each point represents the mean ± SEM of 3–6 experiments performed in triplicate.

TABLE 1.—β-Glucosidase effect on Kemdalin Amigdalina cytotoxicity against AML and normal myeloid colony formation in vitro*

Kemdalin Amigdalina (mg/ml)	β-Glucosidase (μg/ml)	Colonies (% of control ± SEM)		
		HL-60	KG-1	NHBM†
0.5	—	91 ± 3	85 ± 4	90 ± 3
0.5	5.0	75 ± 5	60 ± 4	73 ± 4
1.5	—	78 ± 4	75 ± 6	79 ± 3
1.5	5.0	59 ± 5	46 ± 4	45 ± 3
3.0	—	61 ± 4	55 ± 3	60 ± 2
3.0	5.0	39 ± 5	31 ± 2	38 ± 4
6.0	—	39 ± 4	15 ± 3	20 ± 3
6.0	5.0	5 ± 1	0	0

*Data derived from 3 experiments performed in triplicate. β-Glucosidase at a concentration of 5.0 μg/ml had no inhibitory effect when added to plates containing no Kemdalin Amigdalina.
†NHBM = normal human bone marrow.

mans have been estimated at serum levels of approximately 3 μg/ml (22). We found the myeloid colony-forming cells from human acute leukemia cell lines and normal marrow to be relatively resistant to amygdalin and its metabolites even after 12 days of continuous exposure to the agents.

Drug studies performed in vitro allow a number of variables to be controlled, but the in vitro systems also have important limitations. For example, many drugs are transformed in vivo to active metabolites before they affect the target cell, and drug metabolism to the active compound may not occur in vitro. In this study, we cocultured amygdalin and β-glucosidase to enhance hydrolysis of the parent compound. Direct measurement of percent hydrolysis of amygdalin in vitro, however, was not performed. Preincubation with β-glucosidase markedly increased amygdalin toxicity in vivo and the concentration of amygdalin with β-glucosidase that was lethal to mice is comparable to that previously reported (7,8). Another potential problem with in vitro studies is that cells may be metabolically different in culture than in vivo. This possibility cannot be excluded with the cells from the human AML lines used in this study.

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Sequential Use of Endocrine Therapy and Chemotherapy for Metastatic Breast Cancer: Effects on Survival [1,2]

Andrea Manni,* Jaime E. Trujillo, and Olof H. Pearson [3]

SUMMARY

One hundred and ten patients with stage IV breast cancer were treated with five-drug chemotherapy consisting of prednisone, cyclophosphamide, 5-fluorouracil, methotrexate, and vincristine. Sixty-four percent of 97 evaluable patients achieved a remission, with a median duration of 9 months. Age, disease-free interval, and menopausal status did not affect response to chemotherapy. Patients with visceral dominant site of disease tended to have a lower response rate compared to those with bone or soft tissue dominant site of disease. Remission rate was similar in hormone-responsive and -resistant tumors, although median duration of remission was longer in the former group (11 versus 9 months; $P < 0.05$). Median survival from onset of metastasis was much longer in patients who had responded to previous endocrine therapy than those who had failed (53 versus 23 months; $P < 0.0005$). Thirty-four percent of the 59 patients who were subsequently treated with Adriamycin after either relapse or failure with combination chemotherapy obtained further palliation, with a median duration of 1½ months. We conclude that cytotoxic chemotherapy is effective in hormone-responsive and -resistant tumors. Sequential endocrine therapy and chemotherapy offer long-term survival to patients with hormone-responsive tumors.

[Cancer Treat Rep 64:111–116, 1980]

Endocrine therapy affords effective palliation in about 40% of females with stage IV breast cancer. With the introduction of estrogen receptor (ER) measurements in the primary or metastatic tumor, the response rate to hypophysectomy (1) and to antiestrogens (2) is increased to 65% in those patients with ER-positive tumors, whereas the response rate is virtually nonexistent in patients with ER-negative tumors. Cooper (3), using a five-drug combination chemotherapy, reported a high incidence of remissions (90%) in females with advanced breast cancer. We report here our experience with this combination chemotherapy, with particular interest in the responses of patients who failed to benefit from endocrine therapy versus those who initially responded and then relapsed. We also report our experience with the sequential use of Adriamycin as a single agent after five-drug chemotherapy.

MATERIALS AND METHODS

A total of 110 patients with progressive metastatic breast cancer were treated with combination chemotherapy. Thirteen patients were not evaluable because they died shortly after (< 6 weeks) beginning treatment because of progressive disease (seven patients), pulmonary embolism (three), and gram-negative sepsis, myocardial infarction, and severe gastrointestinal bleeding from peptic ulcer (one each). Thus, 97 of the 110 patients were evaluable. Patients ages ranged from 29 to 80 years. All but three of the females were postmenopausal. Of the 97 patients, 27 had received no previous treatment whereas 71 had previously received endocrine therapy. Among these patients, the dominant sites of disease were visceral (56 patients), bone (32), and soft tissue (nine).

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TABLE 1.—Response to combination chemotherapy and survival in patients with hormone-responsive and -resistant tumors*

Group	No. of patients	Median duration of response to endocrine therapy in mos (range)	Remissions to chemotherapy		Median duration of remission in mos (range)	Median survival in mos	No. of patients still alive
			No.	%			
A: Responders to endocrine therapy	27†	16 (3–42)	20	74	11‡ (4–26)	53§	2
B: Failures to endocrine therapy	39¶	—	25	64	9 (3–20)	23**	2
C: No previous endocrine therapy	26	—	13	50	5 (3–36)	13.5	2

*From onset of metastasis until death.
†Hypophysectomy in 26 patients and adrenalectomy in 1.
‡$P < 0.05$, group A vs B.
§$P < 0.0005$, group A vs B and group A vs C.
¶Hypophysectomy in 22 patients, oophorectomy in 9, tamoxifen in 7, and estrogens in 1.
**$P = 0.005$, group B vs C.

The following regimen was initially used. Prednisone was given orally at a dose of 0.75 mg/kg/day; this dose was reduced by one half every 10 days until it was discontinued after 1 month. Cyclophosphamide was given orally at a dose of 2 mg/kg/day. 5-Fluorouracil (5-FU) at a dose of 12 mg/kg and methotrexate at a dose of 0.6 mg/kg were given iv every week for the first 3 months, every other week for the following 3 months, and every third week thereafter. Vincristine at a dose of 35 μg/kg was also given iv with the 5-FU and methotrexate for the initial six treatments; thereafter, it was given every sixth week. Patients remained in the hospital for the initial 2 weeks of treatment for close monitoring of their blood cell counts. They were then administered the iv treatments on an ambulatory basis. Subsequently, because of severe depression of blood cell counts in many patients, we reduced the doses of the cytotoxic drugs as follows: cyclophosphamide, 50 mg twice daily; 5-FU, 500 mg; methotrexate, 25 mg; and vincristine, 1.0 mg. Because of the high incidence of pulmonary embolism, especially in the early phases of the treatment (*see* below), we later started to use a minidose of heparin (5000 units sc twice daily) during the initial 2 weeks of hospitalization. The dose of the drugs was further reduced in the presence of bone marrow suppression. Vincristine was withheld if neurotoxicity occurred. This combination chemotherapy was continued, with the exception of prednisone, until disease progression was documented. Fifty-nine patients who had either relapsed or failed this combination chemotherapy were subsequently treated with Adriamycin at a dose of 60 mg iv every third week. The total dose never exceeded 500 mg/m^2.

Remission is defined as complete or partial (> 50%) regression of measurable lesions, or recalcification of osteolytic lesions and no new lesions appearing for at least 3 months. Failure is defined as a > 25% progression of measurable lesions or the appearance of new lesions after an adequate trial (at least 6 weeks). No progression is defined as a < 50% regression and a < 25% progression of measurable lesions with no new lesions appearing for a period of at least 6 months.

ERs in the tumors were measured by a dextran-coated charcoal technique (4). Statistical analysis of the data was done using the chi-square or unpaired student's t test.

RESULTS

Response to Chemotherapy in Relation to Endocrine Therapy

Table 1 illustrates the results of chemotherapy in relation to previous hormone treatment. Five patients are excluded from this evaluation because of unevaluable response to endocrine therapy (two patients) and mixed response to hormone therapy (three), ie, response to one treatment and failure to a subsequent treatment. Of the 27 hormone-responsive patients, 26 were treated with hypophysectomy and one was treated with adrenalectomy. Eight of the 26 patients who had responded to hypophysectomy had also previously responded and then relapsed to other endocrine therapy consisting of oophorectomy (four patients), tamoxifen (one), oophorectomy and tamoxifen in sequence (one), estrogens (one), and the combination of estrogens and progesterone (one). Of the 39 hormone-resistant tumors, 22 were treated with hypophysectomy, nine with oophorectomy, seven with tamoxifen, and one with estrogens. Seven of the 22 patients who had failed hypophysectomy had also failed previous endocrine therapy consisting of tamoxifen (six patients) and oophorectomy (one). Dominant site of disease was

TABLE 2.—Results of 5-drug chemotherapy for 97 patients with stage IV breast cancer

	Patients		Median duration of remission in mos (range)	No. of patients still in remission
	No.	%		
Remissions	62	64	9+(3–51)	3
Failures	35	36	—	—

TABLE 3.—Menopausal status and response to 5-drug chemotherapy

Menopausal status	No. of patients	Remissions		Failures	
		No.	%	No.	%
Postmenopausal* < 5 yrs	54	37	69†	17	31
Postmenopausal > 5 yrs	43	25	58	18	42

*Includes 3 premenopausal patients.
†$P = 0.29$.

comparable in hormone-responsive (13 visceral and 14 bone) and hormone-resistant tumors (21 visceral, 14 bone, and four soft tissue). It is quite apparent that our combination chemotherapy was highly effective both in responders and nonresponders to previous endocrine therapy, although the duration of remission was significantly longer in the former group. Of considerable importance, we believe, is the long average survival obtained in patients in group A with the sequential use of optimal endocrine therapy and chemotherapy (table 1). Group C consists of patients with poor prognosis; they were not initially treated with endocrine therapy because of negative ERs (12 patients) and/or very advanced disease. Seventy-seven percent of patients in group C had visceral dominant site of disease compared to 48% in group A ($P = 0.03$) and 54% in group B ($P = 0.06$). It can be seen, however, that even in this poor prognosis group (C), chemotherapy offered significant palliation to one half of the patients.

Sixteen patients with advanced disease were treated with endocrine therapy, 13 with tamoxifen, and three with hypophysectomy after chemotherapy. Fourteen of the 16 patients had been previously treated with other endocrine therapy prior to their chemotherapy. Hormone therapy after chemotherapy induced objective remissions in five patients and arrested disease in two, whereas nine patients were considered failures. This indicates that endocrine therapy can be effective in a significant number of patients with advanced disease who have already been treated with various modalities including hormone therapy and chemotherapy.

Overall Results of Five-Drug Chemotherapy

Table 2 summarizes our overall results with five-drug chemotherapy for the entire group. Five-drug chemotherapy induced remissions in two thirds of the patients, with a median duration of 9 months. Remissions and failures did not differ with respect to median age, 51.5 versus 53 years, or median disease-free interval, 15.5 versus 15 months. Response to chemotherapy was also not significantly influenced by menopausal status (table 3) or by dominant site of disease (table 4), although patients with visceral dominant involvement tended to have a lower response rate. It should be noted that of the 24 patients with bone as the dominant site of disease who responded to chemotherapy, recalcification of osteolytic lesions occurred in 17 patients whereas bone disease remained stable and soft tissue disease regressed in seven patients. Side effects from chemotherapy were usually mild after the dose of the drugs was reduced. These effects included bone marrow suppression and nausea and/or vomiting which were easily controllable with antiemetics, with further reduction in drug dose, or by withholding treatment for a few days. In general, patients tolerated the treatment well without significant morbidity. Of concern, however, was the development of pulmonary embolism in 11 of the 110 patients (10%) which prompted the use of minidoses of heparin during the initial 2 weeks of hospitalization. These episodes occurred within the first few weeks of initiation of chemotherapy in nine patients and after 6 and 11 months in two patients. It is impossible to say whether pulmonary embolism was related to the treatment or the underlying disease.

TABLE 4.—Remissions vs failures in relation to dominant site of disease

Dominant site of disease	No. of remissions/ failures	% of patients responding
Visceral	31/25	55
Bone	24/8	75*
Soft tissue	7/2	78

*$P = 0.067$.

TABLE 5.—Response to Adriamycin in 59 patients after 5-drug chemotherapy

Response	Patients		Median duration of response in mos (range)
	No.	%	
Remissions	16	27	4 (3–13)
No progression	4	7	7.5 (6–14)
Failures	39	66	—

Response to Adriamycin After Five-Drug Chemotherapy

Of the 59 patients receiving the modified Cooper regimen, 32 relapsed and 27 failed; they were subsequently treated with Adriamycin. The results are summarized in table 5. One third of the patients obtained further palliation. There was no significant difference in response rate to Adriamycin between patients who had initially responded to the five-drug regimen and those who had failed the five-drug regimen (22% versus 33%). Side effects from Adriamycin were usually mild and consisted of nausea, vomiting, and bone marrow suppression. None of our patients developed any sign of cardiotoxicity.

Response to Chemotherapy and Estrogen Receptors

As shown in table 6, no difference in the response rate to chemotherapy was observed between ER-positive and -negative patients. In eight of the 17 ER-positive patients, the receptor determination was done just prior to the institution of chemotherapy. Five of the eight patients had been previously treated with endocrine therapy. In one additional patient, ERs were positive after chemotherapy, suggesting that most likely they were also positive at the beginning of treatment. Six of these nine patients obtained a remission with five-drug chemotherapy. The remaining eight patients were treated with endocrine therapy between the time of the receptor determination and the institution of chemotherapy. Four of these eight patients obtained an objective remission. Four patients had multiple ER determinations before and after endocrine therapy and/or chemotherapy; none of these patients converted from ER-positive to ER-negative.

Of the 18 ER-negative patients, 13 had the receptor determination done just before the institution of chemotherapy, four were treated with endocrine therapy between the time of ER determination and chemotherapy, and one had the receptor determination done after chemotherapy. None of the patients in this group had multiple ER determinations.

The accuracy of our ER assay is confirmed by the correlation of receptor status and response to endocrine therapy in two series of patients whom we have recently evaluated. Approximately two thirds of the ER-positive patients responded to antiestrogen treatment (2) and hypophysectomy (1), whereas none of the ER-negative patients responded to either therapy. These results are similar to those already in the literature (5).

DISCUSSION

The introduction of combination chemotherapy, initially by Greenspan (6) and subsequently by Cooper (3), has provided a major improvement in the treatment of metastatic breast carcinoma compared to the use of single-agent chemotherapy. Numerous different combinations of cytotoxic drugs have been found to induce remissions in clinical trials in one half to two thirds of females with stage IV breast cancer (7–9). The overall results presented show that excellent palliation was obtained with our modified Cooper regimen in this group of patients. It should be noted that our patients had very advanced disease as indicated by the fact that the majority had visceral dominant site of disease (table 4). In this regard, the 50% remission rate obtained in a subgroup

TABLE 6.—ERs and response to chemotherapy

ER determination	No. of patients	Remissions		Failures	
		No.	%	No.	%
ER-positive	17	10	59	7	41
ER-negative	18	9	50	9	50

of patients who had particularly poor prognosis (group C, table 1) is remarkable. One aspect of our modified Cooper regimen which deserves mention is the relative lack of significant side effects. Although most females experienced some degree of bone marrow suppression and nausea and vomiting, these side effects were usually easily controlled with appropriate reduction in the dose of the drugs. In this respect, we found it useful to keep the patients in the hospital during the initial 2 weeks of treatment for daily blood cell counts to identify those patients who were particularly sensitive to chemotherapy and who would require special attention as outpatients. Of concern, however, is the high incidence of pulmonary embolism. To the best of our knowledge there are no reports in the literature which associate pulmonary embolism specifically to chemotherapy. It is impossible to say whether this phenomenon is a complication of treatment or somehow related to the underlying disease in this series.

Adriamycin has been shown over the past few years to be a very effective drug in the treatment of breast cancer. Some studies have indicated that drug regimens using Adriamycin in combination may be superior to those which utilize various combinations of cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone (9,10). It has been our practice to use Adriamycin as a single agent after five-drug chemotherapy and, as shown here, we have been able to obtain significant palliation in approximately one third of patients regardless of their previous response to combination chemotherapy. However, it remains to be established whether it is more advantageous to use Adriamycin initially as part of a combination chemotherapy program or as a followup treatment after a five-drug regimen.

Of particular interest is the relationship between response to chemotherapy and previous endocrine therapy. Our data show that the remission rate to chemotherapy was similar in hormone-responsive and -resistant tumors, although the duration of response was significantly longer in the former group. Such results agree with previous reports in the literature showing no relationship between response to prior hormonal therapy and chemotherapy (11,12). In one paper, patients who responded to adrenalectomy had a higher remission rate to chemotherapy than the patients who had failed (13). In these previous reports, however, endocrine therapy and/or chemotherapy are heterogeneous and frequently suboptimal. In contrast, we use optimal endocrine therapy and chemotherapy, thus adding more consistency and validity to the results.

The group of patients with hormone-responsive tumors (group A, table 1) deserves a special comment. This represents a very favorable group of patients and we were able, with sequential use of optimal endocrine therapy and chemotherapy, to obtain long remissions and survival. It is remarkable that the median survival time reaches 4½ years after the onset of metastasis. This group of patients represents the rationale of our philosophy in the treatment of metastatic breast cancer. Unless the patient has negative ERs and/or advanced liver metastasis we try to obtain maximal beneficial effect from endocrine therapy first, because it is usually more readily accepted by the patient. We begin with antiestrogen therapy, tamoxifen, which is highly effective and safe (2). When relapse occurs, we try transsphenoidal hypophysectomy which, under these circumstances, has induced a remission in $> 50\%$ of the patients (14). After relapse from hypophysectomy, we usually try chemotherapy, although in a few selected patients we have observed further remissions with the use of androgens.

Although we obtained excellent results with sequential use of endocrine therapy and chemotherapy, the question arises as to whether better results could be obtained by the combined use of these two optimal treatment modalities. The answer to this important question can only be found by a randomized prospective study.

Finally, we would like to comment on the relationship between response to chemotherapy and ER status. Recently, controversial reports have appeared in the literature concerning the role of ER status in predicting response to chemotherapy. Whereas Lippman et al (15) found that ER-positive patients had a markedly lower response rate to chemotherapy compared to ER-negative patients, Kiang et al (12) reported completely opposite results. In the experience of Kiang et al (12), receptor-"rich" patients had a significantly higher response to chemotherapy than receptor-"poor" patients. Our results, limited however to a small number of patients, indicate that response rate to chemotherapy is equal in ER-positive and -negative patients. It should also be noted coincidentally that in our limited experience hormone treatment did not seem to change the ER status of patients from positive to negative. The reasons for such a discrepancy among different investigators are not readily apparent at the present time. Thus, the role, if any, of ER status in predicting response to chemotherapy requires further investigation.

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Tamoxifen and Fluoxymesterone in Advanced Breast Cancer: A Controlled Clinical Trial[1,2]

Helena Westerberg[3,4,*]

SUMMARY

Seventy-nine postmenopausal women with stage IV breast cancer and no previous endocrine therapy or chemotherapy received tamoxifen or fluoxymesterone in an open, randomized, cross-over trial. The overall remission rate was 30% with tamoxifen as the first course of treatment and 19% with fluoxymesterone as the first course of treatment. Bone metastases were seen in 21 patients receiving tamoxifen and in 20 patients receiving fluoxymesterone. There were six and five remissions in these two groups respectively. The time to the first change in therapy was significantly longer for the tamoxifen group ($P = 0.003$). The survival of the patients who received tamoxifen as the first course of endocrine treatment was better ($P = 0.05$).

[Cancer Treat Rep 64:117–121, 1980]

Recent reports indicate that treatment of advanced breast cancer with the antiestrogen tamoxifen is effective, with response rates of between 30% and 40% and a low incidence of side effects (1). A study by the European Organization for Research on Treatment of Cancer compared the antiestrogen nafoxidine with ethinyl estradiol and showed a better response rate for the antiestrogen (2). No trial comparing tamoxifen to the traditional additive endocrine treatment as expressed by Stoll (3) has been published to date. However, tamoxifen seems to have replaced estrogens in clinical practice probably because of the difference in side effects.

The purpose of this trial was to investigate the differences, if any, in indications for tamoxifen and androgen treatment in advanced breast cancer. Fluoxymesterone was the androgen chosen for the study, because of its long-established use (4,5) and its similarity in administration to tamoxifen (two tablets twice daily).

This randomized prospective study compares tamoxifen and fluoxymesterone in 79 postmenopausal women with advanced breast cancer. Tamoxifen seems to be more acceptable than fluoxymesterone and appears to be at least as efficient in terms of the number of remissions, even in patients with bone metastases.

MATERIAL AND METHODS

The trial was carried out as an open, randomized, cross-over study. Seventy-nine postmenopausal women with progressive, locally advanced, or disseminated breast cancer not previously treated by additive endocrine therapy or chemotherapy were accepted for the trial. Twenty-seven concurrent patients were excluded from the study. The criteria for noneligibility were: (*a*) nonevaluable disease, eg, partially removed local recurrences or pleural effusion as the sole manifestation of disease (eight patients); (*b*) very rapidly progressive disease or massive liver metastases where chemotherapy was the treatment of choice (nine patients); (*c*) complicating diseases, concurrent malignancies, or serious debility due to old age (seven patients); and (*d*) suspected noncompliance with treatment instructions (three patients).

Before randomization the patients were stratified according to two prognostic criteria: disease site and disease-free interval. The site of disease was subdivided into three groups: (*a*) soft tissue, (*b*) bone with or without soft tissue, and (*c*) visceral with or without bone and/or soft tissue.

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TABLE 1.—No. of patients in the study by allocation to first treatment and site of tumor

Site of tumor	No. of patients* receiving— Tamoxifen	Fluoxymesterone	Totals
Soft tissue only	6 (1)	11 (3)	17 (4)
Bone ± soft tissue	11 (3)	11 (5)	22 (8)
Visceral ± bone and/or soft tissue	20 (8)	20 (7)	40 (15)
Totals	37 (12)	42 (15)	79 (27)

*Nos. in parentheses denote patients with a disease-free interval of < 2 yrs before randomization.

The disease-free interval was also divided into two groups: 0–2 years and > 2 years from primary treatment to diagnosis of recurrence. There were thus six groups of patients who were allocated for treatment by random number tables. In order to compensate for the disadvantages of the open design and the subjectiveness of some of the criteria of eligibility, all decisions concerning treatment were made collectively by the five oncologists serving in the breast cancer unit at Radiumhemmet.

Of the 79 patients who entered the trial, 37 began treatment with tamoxifen and 42 began treatment with fluoxymesterone. Table 1 shows the distribution of patients within the treatment groups according to site of disease and disease-free interval. The mean age for patients in the tamoxifen group was 63 years (range, 40–77 years) as compared to 66 years (range, 49–86 years) for patients in the fluoxymesterone group. Of these, three in the tamoxifen group and two in the fluoxymesterone group had had previous oophorectomy because of their disease.

The patients received either tamoxifen (20 mg twice daily) or fluoxymesterone (10 mg twice daily). If the first course of treatment failed to produce a remission or if the disease again progressed after a remission during the first course, the second treatment was begun. No rigid intervals to allow for rebound effects were stipulated between the two courses of medication. If the patient's clinical status had rapidly deteriorated and further endocrine treatment was not advisable, the patient was withdrawn from the protocol and was treated with chemotherapy. There were two chemotherapy regimens: either a modification of the CMF combination (cyclophosphamide, methotrexate, and 5-fluorouracil) proposed by Bonadonna et al (6) with chlorambucil replacing the cyclophosphamide (ten patients) or vincristine, Adriamycin, and cyclophosphamide (eight patients) (7). Subsequent management included progesterone treatment (three patients) and adrenalectomy (two patients).

Baseline Studies and Followup

When admitted to the study all patients had a complete clinical examination including appropriate photographs and measurements by caliper of the two largest perpendicular diameters of up to eight of all palpable or visible tumors. Diagnosis of 43 soft tissue metastases was made by fine-needle aspiration biopsy and was confirmed histopathologically in 24 cases. Roentgenograms of lung fields were taken. Isotopic bone scanning was used to screen for skeletal metastases, and positive evidence was further confirmed by skeletal roentgenograms. Of 41 patients with bony metastases five also had the diagnosis confirmed by fine-needle aspiration biopsy. Liver function tests, serum calcium, and hematologic indices were obtained. Estrogen-receptor analysis was performed on 37 patients as part of the work-up before randomization. Twenty-seven of these analyses were done with surgically obtained material by the method described by Wrange et al (8) and ten were done with tumor material obtained by fine-needle aspiration biopsy (9). Nine of 16 patients (56%) in the tamoxifen group and 12 of 21 patients (57%) in the fluoxymesterone group had estrogen-receptor levels at or above the median of 0.64 femtomols/μg of DNA. The range was 0–3.78 in the tamoxifen group and 0.01–5.50 in the fluoxymesterone group. Clinical evaluation and laboratory tests were repeated at least every 3 months, and roentgenograms and scans were repeated every 3–6 months. Response to therapy was assessed according to the criteria of the International Union Against Cancer (10), which require that time of remission be specified. In order to be classified as a responder a patient had to have a complete or partial remission, objectively verified, lasting for at least 6 months from the start of therapy to the diagnosis of progression.

Results were recorded as remission (> 50% reduction in the sums of diameters of measurable lesions for a minimum of 6 months), no change (< 50% reduction in meas-

TABLE 2.—Response to the 1st course of treatment

Response	No. of patients receiving— Tamoxifen	Fluoxymesterone
Remission*	11	8
No change	6	3
Progressive disease	20	31
Totals	37	42

*Mean remission time = 14.4+ mos (range, 9+ to 21+) for tamoxifen and 10.6+ mos (range, 6+ to 19+) for fluoxymesterone.

urable lesions for a minimum of 6 months), or progressive disease.

The 6-month duration of remission is a result of the 3-month maximum examination interval for some patients. Shorter remissions were disregarded and the patients were classified according to their subsequent response.

RESULTS

Response to the first course of treatment is shown in table 2. The response rate in the tamoxifen group was 30% ± 15% (95% confidence interval). The response rate in the fluoxymesterone group was 19% ± 12%. Three of 20 nonresponders to fluoxymesterone responded with remission after a second course of treatment with tamoxifen and one of ten nonresponders to tamoxifen responded to fluoxymesterone. Not all of the patients have as yet finished a second course of therapy.

When patients are subdivided according to site of disease, as shown in table 1, there are remissions in all tumor groups to either treatment. However, the numbers of patients in the subgroups are too small to allow any valid statistical comparisons. The 18.5% remission rate in patients with a 0–2-year disease-free interval is less than that for patients with a disease-free interval of > 2 years (27%). There is, however, no difference between the two drugs in this respect.

One method of comparing the benefit and suitability to the patients of the two treatments is to measure the time interval to the first change of treatment, irrespective of the treatment results. Figure 1 shows that there was a significantly longer time to the first change of therapy for the patients whose initial treatment was tamoxifen compared to the patients whose initial treatment was fluoxymesterone ($P = 0.003$, log-rank test). After the patients were crossed-over to the second treatment there was again a longer treatment time with tamoxifen compared to fluoxymesterone (data not shown).

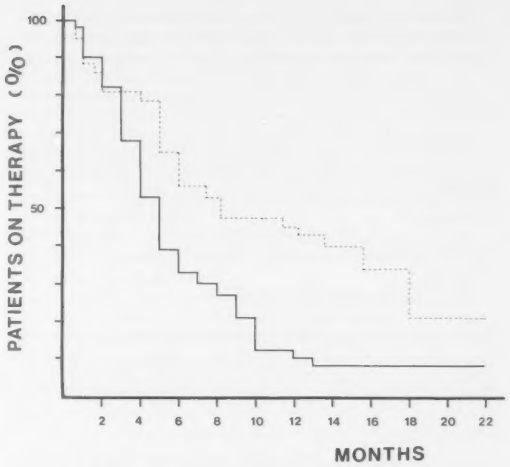

FIGURE 1.—Time to change of therapy. Time interval from start of tamoxifen (broken line) or start of fluoxymesterone (solid line) to first change of therapy. The difference between the therapy change rates is significant ($P = 0.003$, log-rank test).

Six patients in the tamoxifen group have died as compared to 15 in the fluoxymesterone group. The survival rates show a statistically significant difference ($P = 0.05$, log-rank test) (fig 2). Three patients in the tamoxifen group and seven in the fluoxymesterone group died without having changed their treatment. One tamoxifen patient and six fluoxymesterone patients had had a second course of fluoxymesterone and tamoxifen respectively.

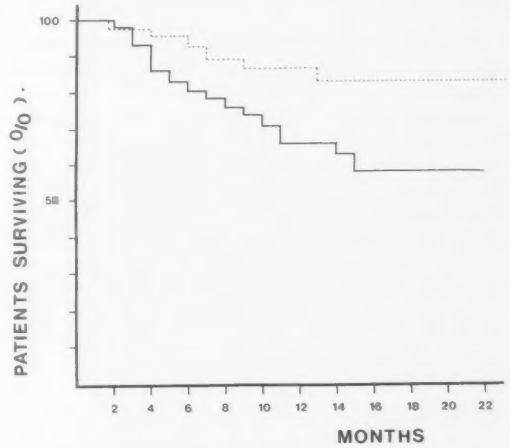

FIGURE 2.—Survival of patients receiving tamoxifen therapy (broken line) or fluoxymesterone therapy (solid line). The difference between survival rates is significant ($P = 0.05$, log-rank test).

TABLE 3.—Results of 1st treatment for all patients with metastatic bone lesions regardless of other tumor loci

Response	No. of patients receiving— Tamoxifen	Fluoxymesterone
Remission	6	5
No change	2	2
Progressive disease	13	13
Totals	21	20

Two tamoxifen patients and two fluoxymesterone patients who died had had further treatment with combination chemotherapy. Among the patients who died none had had further additive endocrine therapy or adrenalectomy.

Since the results of therapy in patients with bone metastases were of particular interest, they are listed separately (table 3). There was a comparable number of patients with bone metastases in the two groups, regardless of involvement at other sites. The pattern of response in these patients is not different from the pattern of the entire patient population. Of the group whose initial treatment was tamoxifen, six of 21 had remission, while in the fluoxymesterone group there were five responders out of 20 treated. All 11 responders had osteolytic metastases that recalcified, with metastases at other sites regressing at the same time. No new lesions appeared at any site.

Side Effects

The side effects were mild in both treatment groups (table 4). As expected there was a marked difference between the two groups in that patients who were given fluoxymesterone for > 3 months all showed some degree of virilization such as hoarseness and increased hirsutism of the face. The most common side effect in the tamoxifen group was hot flashes. The most serious side effect was life-threatening hypercalcemia in two patients with bone metastases. Both occurred within a few weeks of the start of treatment with tamoxifen. Liver toxicity, evidenced by transient increases in enzyme levels, was seen in five patients receiving fluoxymesterone; however, in these patients this side effect was not serious enough to warrant a change in therapy.

DISCUSSION

The effects of estrogens and androgens is believed to be exerted by direct action on the tumor cells. This conclusion is based on the effects of these steroids on cancer cells in culture and on the finding of specific receptors

TABLE 4.—Side effects of tamoxifen and fluoxymesterone

Side effects	No. of patients receiving— Tamoxifen	Fluoxymesterone
Hoarseness and/or hirsutism	—	31
Hot flashes	19	—
Liver toxicity	—	5
Nausea, malaise	1	2
Hypercalcemia	2	—
Hematologic toxicity	—	—
Change in therapy because of side effects	3	3
Total No. of patients receiving drug	62	56

(11). A large number of breast cancers have receptors for estrogen while a smaller number have receptors for androgens (12,13). This may explain the clinical finding that tumor regression is obtained in a greater proportion of patients receiving estrogen therapy compared to androgen therapy (14–17).

Most investigators have found that soft tissue metastases have the highest rate of remission with all kinds of endocrine manipulations, and visceral disease has a lower rate of remission. Among patients with bone metastases Stoll (16) has found a lower response rate (7%) with estrogen therapy than with androgen therapy (19%), but this has not been confirmed (15). However, the response criteria of Stoll are not the same as those of the Cooperative Breast Cancer Group, which may account for the difference in results.

A comparison between androgen and antiestrogen treatment has not been published to date. Our findings of a 30% overall remission rate in the tamoxifen group and a 19% remission rate in the fluoxymesterone group are within the expected ranges (14,16,17). In our patients the number of responders among those with bone metastases is quite similar for the two groups, five of 20 patients for fluoxymesterone and six of 21 patients for tamoxifen, which is comparable to the results recently reported by Manni et al (18). The occurrence of two cases of hypercalcemia among the tamoxifen-treated patients is possibly due to chance since hypercalcemia will occur in about 15% of patients with breast cancer.

Our observation of a prolonged duration of therapy with tamoxifen both as the first and the second course of treatment is not entirely explained by longer durations of remissions, since we also observed longer treatment times in the nonresponder category with this drug. Remission times with estrogen treatment are longer than with androgen treatment (13). The longer treatment

times for tamoxifen argue for a greater suitability of this drug. We also observed a prolonged survival time in the tamoxifen group ($P = 0.05$). This result must be interpreted with caution because of the small number of patients and the 3 years' difference in mean age between the two treatment groups. Our conclusion is that there is no obvious advantage to the postmenopausal woman with disseminated breast cancer to start her additive endocrine treatment with androgen regardless of bone involvement. This would spare or at least postpone for many patients the distress of virilizing symptoms.

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Quantitative Changes in *cis*-Dichlorodiammineplatinum(II) Speciation in Excreted Urine With Time After Iv Infusion in Man: Methods of Analysis, Preliminary Studies, and Clinical Results [1]

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SUMMARY

Chemical species containing platinum originating as *cis*-dichlorodiammineplatinum(II) (CDDP) were isolated, quantitated, and characterized operationally as a function of time from quantitative urine collections from cancer patients receiving CDDP via iv infusion for the first time. Before these clinical studies, similar measurements were made from (*a*) infusion solutions of CDDP in vitro and (*b*) urine collected from healthy subjects and incubated in vitro with CDDP. The platinum species in all urine excreted by a patient during the drug infusion and for 48 hours after the infusion were fractionated by successive elutions from a cation-exchange resin. They appeared as single peaks in each eluate and as such were operationally defined as: A, water-elutable platinum species, and B, HCl-elutable platinum species. Each species A and B exhibited very similar elution characteristics when compared with those of the same species at 1.8 and 24.4 hours after the start of the 1-hour infusion in patient 1 and at 3.7 and 20.4 hours after the start of the 6-hour CDDP infusion in patient 2, which suggested that they were probably chemically unchanged over at least this time interval. However, the relative concentrations of A and B and the total concentration of platinum changed markedly with time. The proportions of the platinum species A and B present in excreted urine remained nearly constant during the 6-hour drug infusion at the values found in the aging infusion solution in vitro. After the CDDP infusion was completed, the proportions of A in the urine declined progressively from 87% to 15%; there was a nearly concomitant increase in the proportions of B from approximately 13% to 60% over the next 7 hours. A third fraction not present initially but increasing in concentration with passage of time after the start of the drug infusion stayed tenaciously bound to the resin. The proportion of A in the successive urine samples collected after the end of the drug infusion declined, obeying a first-order rate law with a half-time of ~1.8 hours. The several forms of platinum continued to be excreted in the patient's urine until the end of the study. The progressive speciation that occurred on in vitro

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incubation of CDDP in two urine samples (urine sample 1 with [Cl^-] = 0.060 M and pH 5.0; urine sample 2 with [Cl^-] = 0.124 M and pH 5.67) collected from healthy subjects produced the same operationally defined species A and B but in different proportions. The above findings are consistent with the chemical model involving hydrolytic equilibria, proposed in our earlier work. They show that water-elutable and acid-elutable species change in their quantitative predominance over time and suggest that the platinum species content in these urine samples is related to the chloride ion content of the urine.

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cis-Dichlorodiammineplatinum(II) (CDDP), a coordination compound of divalent platinum, is being used for cancer chemotherapy in clinical trials. Several studies have described the distribution of platinum coordination compounds in the body following their administration to animals (1–6) and to man (7,8). Most reported studies have followed the distribution of total platinum species, making no distinction between those that are protein-bound and those that are "free," nor between the individual platinum species that obtain in the "free" population.

The ability to distinguish individual species or groups of closely related species is essential to a better understanding of the most effective use of the drug. There is evidence that the bound species do not contribute directly to the drug's therapeutic activity (8) but may serve as a significant reservoir. Clearly, binding at the target site is important. If the drug exhibits a therapeutic effect only in the unbound form, pharmacokinetic analysis should include information on the relative populations of the relevant "free" species present.[5]

Species that are sufficiently slow to react ("inert") can be isolated and quantitated in some cases through use of an appropriate combination of separation and analytic techniques: centrifugal ultrafiltration for separation of bound from "free" species in plasma; ion-exchange chromatography of the resultant plasma ultrafiltrate or of urine for the fractionation of the "free" species in these fluids; and subsequent flameless atomic absorption spectrophotometry (FAAS) for the quantitation of platinum in the several fractions.

In the research reported here, these methods were applied to CDDP infusion solutions, to urine collected from healthy subjects and incubated with CDDP in vitro, and to samples of quantitative collection of all urine from two patients during initial treatment by iv infusion of CDDP and for times up to 48 hours following the infusion. This represents another departure from most earlier reports which presented pharmacokinetic data only for iv bolus administration of CDDP.

MATERIALS AND METHODS

Resin and Column Preparation and Preliminary Experiments

Conditioning of Resin

The fine particles of ion-exchange resin were removed by decantation, and the bulk of the resin was conditioned for use by soaking alternately in fresh solutions of 2 M NaCl and then 2 M HCl through at least six cycles of resin expansion and contraction to free it of impurities, both organic and inorganic. The resin was left in the hydrogen form.

Preparation of the Columns

Pyrex columns were soaked in hot aqua-regia for several hours, and then rinsed thoroughly with deionized water. Virgin resin was loaded into them in the hydrogen form, backwashed, and then flushed with several bed volumes of platinum-free water until the effluent was basic to methyl-orange. The columns were capped and stored until needed.

Preliminary Ion-Exchange Experiments

Fractionation of CDDP infusion solution aged in vitro for various times.—Infusion solutions of the same drug lot of CDDP were prepared at the same concentrations

[5]The terms "unbound" and "bound" applied to the platinum species in a living system could become a misleading oversimplification. In the usual sense, "bound species" may well refer to operationally defined species, eg, those retained by a given ultrafiltration membrane. The metal complex can bind to a variety of nucleophiles, both large (macromolecules) and small (amino acids, peptides, and inorganic species). Most of the latter may pass through an ultrafiltration membrane with a 50,000 molecular weight cutoff. For a given metal complex species, the stability constant that describes the relative proportions of that complex and its conjugate and the nucleophiles (ligands) present at equilibrium (a thermodynamic state) may be large, indicating the formation of a high proportion of that complex, and yet it may still be labile (a kinetic property) and be able to shift from that species to another, perhaps unbound, rapidly. In other situations, a complex may be "inert" or "robust" in the parlance of coordination chemists, and the change occurs only slowly.

and in the same sterile medium as used in treating the patients and were stored at ambient temperature in the laboratory (20°C–22°C). Duplicate samples of such solutions were withdrawn at various times after preparation and fractionated by the ion-exchange procedure applied in the clinical study detailed below.

Fractionation of platinum species in urine collected from healthy subjects and incubated with CDDP for various times.—CDDP was added to urine collected from two healthy subjects. Aliquots containing CDDP at initial total platinum concentrations in the same range as those excreted by patients ($\sim 10^{-5}$ M) were aged in tubes held in a thermostatted bath at 37°C. Samples were withdrawn at intervals up to 48 hours for fractionation and analysis. Remaining samples were stored for measurement of the "infinite time" distribution 2 weeks later. Elution characteristics of the platinum-containing species were established by collecting and analyzing successive fractions of eluates. Initial studies with eluant fraction volumes of 1–2 ml gave evidence of single peaks in each eluate. These were confirmed by subsequent experiments with fractions of 0.25 ml and total eluant volumes of 15–25 ml. Two such studies made in some detail at different pH and pCl revealed that under the conditions obtaining, each eluate contained one predominant platinum species.

Platinum Analysis by FAAS: Construction of Elution Curves

In both the preliminary and the clinical studies, a portion of each sample, aged for a given time, was analyzed for total platinum by FAAS to provide a basis for estimation of platinum species retained on the resin after elutions. The platinum content of each eluate is reported as a fraction of the total platinum in a sample not contacted with the ion-exchange resin. The elution characteristics of the platinum-containing species in these aged samples were established for each time point by quantitating the platinum in small fractions of each eluate collected seriatim from the ion-exchange column. The water-elution curve and HCl-elution curve were constructed for each. Details of the platinum analysis technique have been reported elsewhere (9).

Clinical Studies

Patients

The two male patients studied were receiving CDDP chemotherapy for the first time. Both patients had an expected survival of > 6 weeks; they both had histologically proved testicular cancers. They had patent lower urinary tracts as demonstrated by iv pyelogram and by rectal examination that would have revealed any benign prostatic hypertrophy, and their renal function was normal as indicated by a BUN of < 20 mg/100 ml, a serum creatinine of < 1.5 mg/100 ml, normal urinalysis, and a creatinine clearance of > 70 ml/minute (range, 70–90 ml/minute). All blood values, including protein levels, were within normal limits, indicating no manifest organ abnormalities. Patient 1 had recovered from the toxic effects of prior chemotherapy (minimum elapsed time of 3 weeks). Patient 2 had received no prior chemotherapy. Each had a wbc count of > 4000/mm^3 and a platelet count of > 130,000/mm^3.

Both patients were hospitalized and prehydrated prior to infusion of the CDDP. The drug infusion was followed by infusion of a saline solution.

Patient 1.—Patient 1 was treated in the Clinical Center, National Institutes of Health (Bethesda, MD). He received a dose of 100 mg/m^2 of CDDP by a 1-hour infusion. The drug infusion solution was prepared by reconstituting the lyophilized CDDP (supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) with sterile 5% dextrose + physiologic saline and diluting to a total volume of 500 ml. Urine samples were collected as they were voided and were carried to the analytic laboratory of the Biomedical Engineering and Instrumentation Branch, Division of Research Services, in a nearby building for the ion-exchange chromatographic separation step and subsequent analysis.

Patient 2.—Patient 2 was treated by the Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute (Baltimore, MD). He was prehydrated 3 hours prior to drug treatment with 1000 ml of 0.154 M NaCl + 0.020 M KCl. At "zero" time (1 PM on the first day of treatment with CDDP), he started receiving a dose of 131 mg of CDDP (lot No. BV-78-238) at 75 mg/m^2 of CDDP administered by iv infusion of a solution prepared approximately 2 hours before the start of the infusion from the reconstituted drug added to 2 liters of 5% dextrose solution + 0.077 M NaCl + 375 ml of 10% mannitol solution. The iv solution was administered to the patient over a period of 6 hours at a constant rate by means of an IVAC-600 volumetric pump. (The rate of administration for patient 2 thus differed eightfold from that for patient 1, ie, from 100 mg/m^2/hour to 12.5 mg/m^2/hour.) The drug infusion was followed by a 4-hour infusion of 1 liter of 5% dextrose solution + 0.077 M NaCl at a constant rate of administration (the same pump was used). For this study, the urine samples were carried to an adjoining hospital room, where the chromatographic separations were performed. The procedural details, though similar, were not identical for the studies with patients 1 and 2 (*see* Results and Discussion section); the detailed methods described below are those from the study with patient 2.

Urine Collection, Sample Preprocessing, and Ancillary Measurements (Patient 2)

Within 5 minutes after every excretion of urine, its volume was measured and recorded, and a 10-ml portion

of the urine was filtered by use of a Millipore MF HA (0.22μ pore size) filter. This filtrate was collected for analysis of total platinum and measurement of pH and [Cl^-]. The latter measurements were made on each sample on a timely basis while elutions of the ion-exchange resin were being conducted (*see* below). The pH was measured by use of a Thomas Universal pH Electrode (catalogue No. 4094-L15) versus saturated calomel electrode. Chloride ion concentration was measured in duplicate samples by means of a potentiometric titration (Ag/AgCl electrode versus saturated calomel electrode through a nitrate salt-bridge) with a standardized silver nitrate solution.

Ion-Exchange Fractionation of Platinum Species in Urine (Patient 2)

Column preparation, sample loading, and general fractionation conditions.—Twenty-eight columns (5 mm internal diameter × 30 cm), equipped with fittings suitable for fluid flow control and/or caps, were prepared the day before the patient was treated with CDDP. Each column was loaded with an equal quantity of virgin resin from the same batch. Within 1 minute of the end of the filtration described above, two 1.0-ml portions of the urine filtrate were withdrawn and immediately loaded onto each of two of the previously prepared columns. Fractionations were conducted at ambient temperature; no attempt was made to quench the reactions taking place by chilling the columns or the solutions flowing through them. Duplicate simultaneous chromatographic fractionations were made using 2 ml of cation-exchange resin (Dowex 50X8, 200–400 mesh) initially in the hydrogen form [H^+] in each column. To ensure that the fractionation of species in each urine sample was independent from all others yet comparable with them, the flow of sample and eluants through each column was controlled at a rate of 1 ml/minute by means of a Buchler Polystaltic 2-1600 pump. As the first 28 resin samples were collected, the columns made available were soaked in aqua-regia, rinsed, reloaded with fresh resin, and stored until needed for use again.

Elution.—In order to be able to separate the platinum species with no more than a few minutes' delay after the urine was voided, and to keep the number of platinum analyses required within reason, samples were loaded on the column and eluted with a fixed volume of water (20 ml) and then with a fixed volume of 6 M HCl (20 ml) each collected separately. With this integral elution procedure, it was possible to keep pace with the excretion of urine by the patient, ie, approximately one sample per hour during the day. Once the fractions had been collected, they were stored for subsequent evaporation, reconstitution with HCl to known volume, and quantitation of the platinum in each by FAAS (9). The resins were transferred to individual vials for storage and, ultimately, for analysis to measure platinum retention in those cases requiring it. In the course of this clinical study, separation of platinum species in three urine samples with mean collection times of 3.7, 20.4, and 50.2 hours after the start of the CDDP infusion was made using the same total eluant volumes, ie, 20 ml each of H_2O and HCl, but with collection and analysis of successive 1.0-ml fractions of each eluate. Eluate fractions were collected by means of two LKB Model 7000 Ultrorac fraction collectors. Because experience with CDDP incubated in urine from healthy subjects revealed no multiple peaks with fractions of 0.25 ml, 1.0-ml fractions were collected in the clinical study to reduce the analytic work required. The three fractional elutions were interspersed with the integral elutions to confirm the conjecture from the study with patient 1 that there was but a single platinum species in each of the two eluates at each different time in the clinical study. (*a*) *First elution:* Immediately after the sample had been loaded on each column and its liquid level had reached the top of the resin, the flow of the first eluant (triple-distilled, deionized water) was begun at a flow rate of 1 ml/minute. This elution was ended when a total sample of 20 ml had been collected for subsequent analysis. This eluate thus contained the interstitial solution in the resin column initially (platinum-free water, ~ 1 ml) and the water-elutable platinum species together with the excess eluant. (*b*) *Second elution:* The second eluant used was 6 M HCl which was started flowing through the resin column at 1 ml/minute immediately after the end of the water elution. A total of 20 ml of the acid eluate was collected in a separate vessel and set aside for later analysis.

RESULTS AND DISCUSSION

Preliminary Studies

Fractionation of Platinum Species in Infusion Solutions

The platinum species distribution in the infusion solution becomes stable within about 4 hours after dissolution in sterile medium maintained at ambient temperature. The low pH of this solution suppresses the formation of possible hydroxo complexes. The relatively high chloride concentration favors the water-elutable form A, which we conjecture is the unchanged CDDP. Two species, A and B, obtain in proportions that are approximately 85% water-elutable and 15% chloride-elutable respectively (table 1).

Fractionation of Platinum Species in Urine Collected From Healthy Subjects

Analysis of fractions of platinum species in samples of urine collected from healthy subjects and incubated with CDDP at 37°C indicated that there was but a single platinum peak in each eluate at each time. There was a progressive decrease in the proportion of the water-elut-

TABLE 1.—Fractionation experiments on infusion solution

Sample	Temperature (°C)	$[CDDP]_0$ (M)	$[Cl^-]$ (M)	pCl	pH	% A at equilibrium	% B at equilibrium	Time required to attain equilibrium (hrs)
Infusion solution	Ambient: 20–22	2×10^{-4}	0.077	1.114	< 4	85	15	< 4

able platinum species A and a corresponding increase in the HCl-elutable species B with time until an equilibrium was attained. The progression is shown in figure 1. The time shown as infinity in the figure corresponds to 2 weeks. Table 2 shows the relevant conditions of the experiments.

These distributions are consistent with an approach to a hydrolytic equilibrium. They point up a rather marked dependence of the behavior of platinum species on other constituents of the solutions and their concentrations (10). The presence of other unmeasured nucleophiles in the urine samples may also play an important role in the speciation observed.

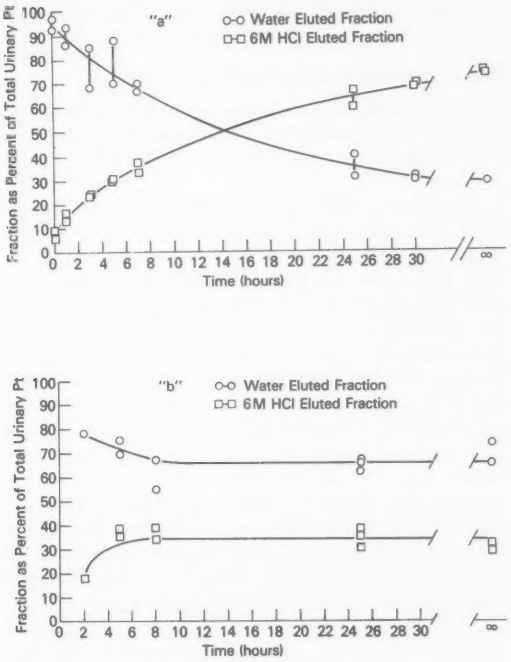

FIGURE 1.—Fractionation of platinum species in urine collected from healthy subjects and incubated at 37°C with CDDP, as a function of time. Zero time = start of incubation. (a) pH 5.0, $[Cl^-] = 0.060$ M; (b) pH 5.67, $[Cl^-] = 0.124$ M.

Clinical Studies

General Comments

The frequency of micturition was highly variable and unpredictable for both patients; during the day urine was excreted at about 1-hour intervals, at times the intervals decreased to ½ hour, and at night they increased to several hours. Difficulties encountered in obtaining the fractionation data in the study with patient 1 prompted numerous changes in procedure. In particular, results obtained in the first study emphasized the need for rapid, timely sample preprocessing and availability of multiple chromatographic setups ready for immediate use when each urine was excreted.

Results obtained from the study with patient 1 are somewhat fragmentary because of (*a*) variable delays in obtaining the urine samples for fractionation, (*b*) variations in handling those samples before analysis (eg, some were refrigerated, some were not), and (*c*) release of the patient from the hospital (at his request) earlier than originally anticipated, curtailing the study and making collection of later samples impossible. Thus, while results for both patients are in agreement in their significant features, the first study suffered from inadequate control of some variables. More complete results were obtained in the second study where, in addition, samples were run in duplicate thus allowing the reproducibility of the measurements to be evaluated. The results of the study with patients 1 and 2 were entirely consistent. Although the former were fragmentary, they served to improve the latter.

General Observations (Patients 1 and 2)

The total concentration of platinum species in the excreted urine followed first a rapid increase to maximum value in the sample collected nearest the end of the infusion, and then a short but rapid decrease followed by intervals of significant rise and fall in platinum concentration that really reflect the diurnal variation in output of urine. The total platinum concentration is shown in figure 2 together with the chloride concentrations ($pCl = -\log_{10}[Cl^-]$) and the $pH = -\log_{10}[H^+]$ of the samples from patient 2. The results of all determinations are referred to the midpoint of the time interval in which the sample was collected.

Ion-exchange chromatography of the platinum species in the urine showed a progressive change in concentrations of species over a time period of approximately 7

TABLE 2.—Fractionation experiments on urine collected from healthy subjects and incubated with CDDP

Urine sample	Temperature (°C)	$[CDDP]_o$ (M)	$[Cl^-]$ (M)	pCl	pH	% A at equilibrium	% B at equilibrium	Time required to attain equilibrium (hrs)
1	37	1.66×10^{-5}	0.124	0.907	5.67	65	35	~8
2	37	3.33×10^{-5}	0.060	1.222	5.0	29	73	> 30

hours after the end of the infusion. The predominance of the single water-elutable species during the infusion shifted to a predominance of a species that was elutable with an NaCl or HCl solution within the several hours following the end of the infusion. Though the fractionated platinum species in excreted urine differed markedly in total concentrations and proportions present at 3.7 and 20.4 hours after the start of the infusion, they exhibited very similar elution behavior, suggesting that the species were probably the same chemically at both times (fig 3). The acid-elution curve has a relatively long tail in all cases. Changing eluants and/or elution conditions to effect a more efficient second elution may resolve more than one species, but our efforts to achieve this result were not successful. The urine sample at 50.2 hours contained the smallest amount of total platinum of all samples in this study. Fractionation of platinum species in it yielded very low concentrations and thus the results obtained for construction of the elution curve contain large uncertainties, though the same species obtain.

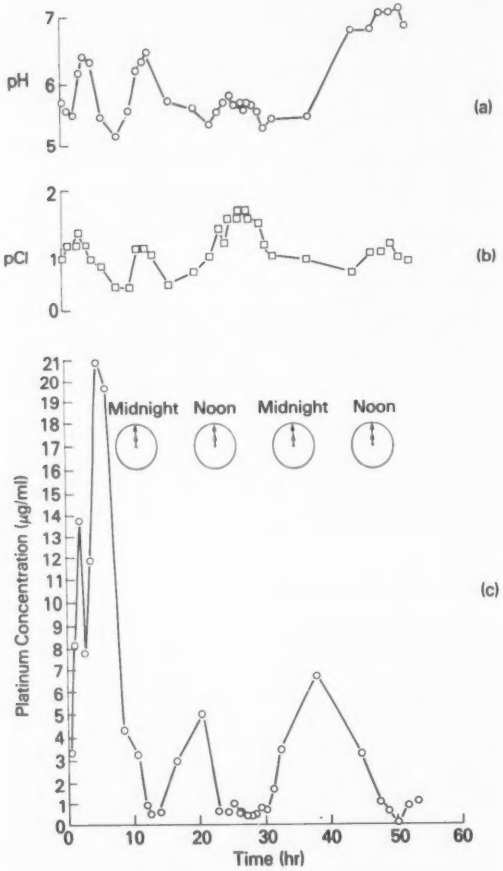

FIGURE 2.—Platinum concentration in excreted urine (curve c) as a function of time for patient 2 and corresponding urinary pH (curve a) and pCl = $-\log[Cl^-]$ (curve b) values. Zero time = start of infusion.

Fractional Elution Studies (Patients 1 and 2)

The fractional elution studies revealed that there was but one peak in each of the two eluates. This was taken as evidence that a single platinum species predominates in each.

Quantitation of the platinum concentration in the 1-ml fractions of the water eluate revealed a peak concentration occurring in the range of 2–3 ml, accumulated volume. This represented approximately 80% of the measured total platinum in the urine samples at 3.7 hours. The platinum concentration fell below the detection limit (approximately 67 ng of platinum on analysis of unconcentrated urine) by the time a volume of 8–9 ml of eluate had been accumulated. Figures 3a and 3b show these results for the urine samples from patient 1 at 1.83 and 24.4 hours and figures 3c and 3d show these results for patient 2 at 3.7 and 20.4 hours after the start of the infusion. For figures 3a and 3b, the water eluates contained 86% and 7% respectively of the total platinum in the urine in a single peak while the acid eluate contained 14% and 93% of the total in a single peak. For figures 3c and 3d, the corresponding quantities were 87% and 13% and 12% and 50% respectively. These percentage values were based on a third, independent analysis of total platinum species in the corresponding unfractionated urine. The fractionation results at the 3.7-hour time point are in very close agreement with those obtained on fractionation of a sample of the infusion solution of CDDP itself 4 hours after preparation and held at room temperature.

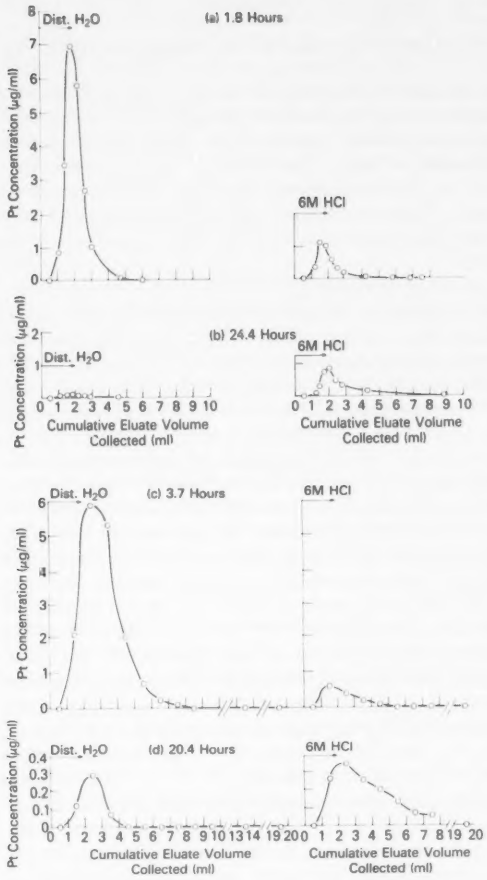

FIGURE 3.—Successive elution curves for platinum species in urine samples from patient 1 (1-hr infusion; zero time = start of infusion) at (a) 1.8 hrs and (b) 24.4 hrs; and for patient 2 (6-hr infusion; zero time = start of infusion) at (c) 3.7 hrs and (d) 20.4 hrs.

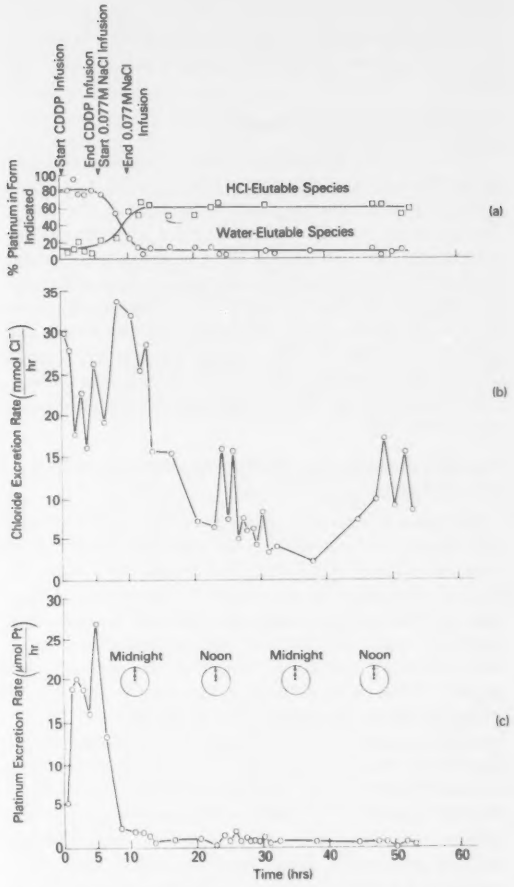

FIGURE 4.—(a) Proportions of operationally defined species A and B present in excreted urine samples (patient 2) as a function of time (average of 2 values). Zero time = start of infusion. (b) Rate of excretion of chloride ion (mmols/hr) excreted in urine as a function of time. (c) Rate of excretion of total platinum species (μmols/hr) excreted in urine as a function of time.

Platinum concentrations of tail fractions fell rapidly to values below the detection limit of the analytic method used. In every instance, the concentration fell below the limit of detection when 10–15 ml of eluate had been collected. It was assumed that collection of 20-ml eluate volumes would include all the detectable water-elutable platinum species in every urine fractionation in the study. The validity of this assumption was confirmed in several samples taken throughout the course of the entire study by collecting the last 4.0 ml of eluate separately, concentrating it by evaporation, and analyzing the resulting solution for platinum. Such solutions contained < 1% of the total platinum in the corresponding samples loaded on the column.

Though the overall time required for the completion of the ion-exchange fractionation step for each sample was approximately ¾ hour (3–5-minute prefiltration + 1-minute loading + two successive elutions of 20 minutes each), it is estimated that the cationic platinum species were separated from the other platinum species in each urine sample by ion-exchange within 5–10 minutes after excretion (3-minute filtering + ~1-minute loading on column + first few minutes of water elution). Since the kinetics of ligand exchange reactions of most platinum

complexes is slow (half-times ~ hours), this procedure was deemed adequate to yield meaningful data on relative species population distribution at times indicated in these studies.

Integral Elution Studies (Patient 2)

The running of duplicate chromatographic separations simultaneously for each sample was a useful measure to assess reproducibility of results throughout the study. The results obtained for each time point were in excellent agreement (usually within 10%), especially for those cases where the platinum concentrations were well above the detected limit of the analytic technique. At times after ~ 24 hours, when platinum concentrations had fallen to very low absolute values, the relative error in the platinum determinations became larger, thus increasing the uncertainty of those results, though most were within 10%–15%.

Changing Proportions of Platinum Species in Urine With Time (Patient 2)

The rates of excretion of total platinum (μmols/hour) and chloride ion (mmols/hour) calculated for samples from each collection of urine excreted by patient 2 after the start of drug infusion are shown in figure 4. The time scale is on the abscissa; other relevant events are marked relative to this scale. The platinum content of each of the two eluates (A and B) is plotted on the ordinate as a percent of the total platinum in the sample obtained by an independent determination in figure 4a.

0–5 Hours after start of CDDP infusion (infusion period).—It is important to note that even in the infusion solutions speciation was evident. Comparison of proportions of species that obtain in a sample of the infusion solution with those in the urine samples collected during the infusion showed considerable similarity. During the course of the CDDP infusion, the percentages of water-elutable and HCl-elutable platinum species remained nearly constant (~ 85% and ~ 15% respectively) while at the same time the total platinum concentration in the urine was rising and peaking.

5–13 Hours after start of CDDP infusion (transition period).—The rate of platinum excretion in the urine voided in the first hours after the end of the drug infusion fell sharply. In the first urine excreted after the end of the drug infusion, the proportions of platinum species started changing, with a progressive diminution in concentration of the water-elutable fraction and an increase in the HCl-elutable portion in subsequent urine samples. This progression continued until approximately 13 hours had elapsed. By that time, the species' proportions had approximately reversed themselves so that the water-elutable fraction then contained approximately 15% of the platinum in the sample. The proportion of water-elutable species in the urine samples excreted after the drug infusion had ended decreased, obeying a first-order rate law with a half-time of 1.8 hours. The half-time of the acid hydrolysis (aquation) step of CDDP

$$cis\text{-}[Pt(NH_3)_2Cl_2] + H_2O \rightleftharpoons cis\text{-}[Pt(NH_3)_2(H_2O)Cl] + Cl^-$$

is estimated to be 1.9 hours at 37°C from the data in the literature (5). The similarity in rates of these two processes is probably fortuitous, as there are many pharmacokinetic factors that differ in the two cases. More work is necessary to clarify the relationship of these processes. These results also suggest that enzymatic processes are probably not involved in the processes occurring.

13–54 Hours after start of CDDP infusion (post-transition period).—Samples collected at later times (13–15 hours after the start of the infusion) contained very low total concentrations of platinum. The results from this later post-transition period indicated that excretion of two or more platinum species continues at a rate about equal to that observed at 13 hours. The proportions indicated that there continued to be approximately 10%–15% associated with the water-elutable fraction in these later urine samples. The total elutable platinum diminished progressively, indicating that some portion of the platinum becomes bound to the resin or is bound to other species (proteins, peptides, or amino acids) in the urine that bind to the resin or become denatured in the resin during elution. This portion is very tenaciously bound, perhaps irreversibly so, and though several eluants were tried, none removed this fraction of platinum. Chemical destruction of such resin samples to recover the platinum was undertaken; the analyses showed that some platinum in urine samples collected after ~ 24 hours did remain in the resin. The quantitative results of these analyses were of the correct magnitude to close the material balance. The accuracy and precision of these determinations was limited, however, by the small absolute quantity of platinum present and by the nature of the experiment.

Plasma Studies

Plasma samples were collected from patient 2 throughout the course of this study. Because circumstances did not permit us to analyze the platinum in these samples at the time of collection, the samples were frozen and stored with dry ice. The samples were later thawed and analyzed, but it was not possible to achieve reproducibility in the platinum analyses. Determinations of platinum in plasma from other patients treated later under the same protocol were reproducible within the limits of our past experience with plasma samples, ie, ±15% accuracy. These showed a rapid rise and then a slow decline over a period of days. The platinum in ultrafiltrates of these same plasmas exhibited a very rapid decline; within 8 hours after the end of the infusion, the concentrations were below our detection limit. Similar rapid declines have been reported by others (8).

Possible Mechanisms Accounting for Observed Urinary Excretion Behavior of Platinum Species

The results presented, being data for platinum in excreted urine, necessarily reflect only distantly the fundamental chemical processes occurring at the level of glomerular filtration or tubular transport (reabsorption or secretion) of the platinum species bound and unbound with other ionic and molecular species in the kidney. The clinical studies do show that at least two platinum species are present that can be cleared by the kidneys for at least some 50 hours after the infusion solution starts entering the body. They are probably not bound to large molecular species.

During the infusion of the CDDP, there is a relatively rapid accumulation of platinum species (A and B) in the body. The finding that, during the infusion, the fractions of A and B appearing in the urine are in proportions very nearly those in the solution being infused indicates that they are both cleared and at clearance rates sufficient to preserve the proportions of each species in the urine to those in the infusion solution.

In the hours immediately following the CDDP infusion, the amount of species A (μmols) in the body is diminished by at least two mechanisms: (*a*) chemical transformation to species B (and other species, *see* below) that may themselves be retained or excreted and (*b*) excretion in the urine as species A.

The cumulative urinary excretion of all platinum species during the infusion accounts for approximately 18.5 mg of platinum (~ 22%) of the 85 mg of platinum infused. Since the A species constitutes 85% of the platinum in the CDDP infusion, approximately 18.5% of the infused A species is excreted in this time.

The proportion of B species in the urine increases with time during the decline of the A species and remains high (but not as high as the original fraction of A) throughout the remainder of the experiment while the proportion of A remains low during that time.

An interpretation of the numerical results and facts presented above is that the A species is unchanged *cis*-$[Pt(NH_3)_2Cl_2]^0$ (of course, since A is operationally defined, it could also include other uncharged species [eg, D and F in reference 5] and negatively charged species) and the B species is the cation *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$.

The first-order rate (half-time = 1.8 hours) of disappearance of species A in urine samples collected after the end of the drug infusion compares closely with the estimated rate of acid hydrolysis of CDDP in vitro at 37°C (half-time = 1.9 hours). Concomitant with the rapid decline of proportions of species A in successive urine samples is a nearly corresponding increase in the proportion of species B (fig 4a). This mechanism could account for disappearance of a large fraction of species A in the first few hours after the infusion ends. Estimates of cumulative excretion of species A and species B from the time species A reaches its minimum proportion in the urine suggest that the A species is probably replenished in the urine from other equilibria, presumably those of bound platinum. Such a system could provide a continuing supply, over a period of days, of species A that is thought to be the favored form for transport into the target cells and could explain its presence in urine in nearly constant proportion to species B for many hours after the infusion was completed.

The pH of urine samples remained low enough throughout the study to permit us to expect little deprotonation of the chloro-aquo species (B) to the corresponding chloro-hydroxo species, at least in the urine during the first 53 hours of the study. Both NaCl and HCl solutions can elute the B species from the resin, suggesting that the Na^+ or H^+ displaces the B species from the resin. Of course, the high $[Cl^-]$ present in the eluate also reverses the hydrolytic reaction, replacing the aquo ligand with a chloro ligand (anation reaction).

$$H_2O + \text{cis-}[Pt(NH_3)_2Cl_2]^0 \rightleftharpoons \text{cis-}[Pt(NH_3)_2Cl(H_2O)]^+ + Cl^-$$

or

$$A + H_2O \rightleftharpoons B + Cl^-$$

thus converting the B platinum species to the A species, again making it easily removable from the resin. We expect the displacement of the complex from the resin to be relatively rapid compared with the chloro-substitution reaction in the complex. Efficient elution probably requires both reactions to occur.

In addition to species A and B, there appears to be formation of at least a third platinum-containing species. Whatever its composition, the third form of platinum that appears in the urine at later times is retained on the resin very strongly and is probably irreversibly bound to it or to another nucleophile such as a protein that is very strongly held to the resin. Such a platinum-binding protein could be denatured while in the resin by the passage of the relatively concentrated eluant, and thus made unelutable. We know that platinum species bind slowly to plasma proteins (5). This would account for the absence of this fraction at early times.

The urine from the healthy subjects is effectively an ultrafiltrate and can thus be expected to contain little, if any, protein. Since the total platinum species concentrations throughout the studies with these normal urine samples remained constant and relatively high compared with measurements made in the clinical study at similar times, it is not surprising that the third type of platinum species was not observed as a major constituent in those samples.

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Pilot Study of Vinblastine and Bleomycin Combinations in the Treatment of Metastatic Melanoma [1,2]

Larry Nathanson* and Barbara Kane Wittenberg [3]

SUMMARY

Fourteen patients with advanced metastatic malignant melanoma were entered in a study with a combination of vinblastine and bleomycin (11 patients) or with this combination plus *cis*-dichlorodiammineplatinum(II) (three patients). Objective partial responses were seen in four of nine evaluable patients receiving vinblastine and bleomycin and in all three evaluable patients receiving vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II). Although there was significant toxicity, it appeared tolerable, and no drug deaths were reported. The three drug-combination may produce less severe toxicity while maintaining a high response rate.

[Cancer Treat Rep 64:133–137, 1980]

Because of the failure of current clinical chemotherapy trials to produce increasing benefit in metastatic melanoma, a more effective drug combination has been sought. Mabel et al (1) reported cure as well as increase in survival of C57BL mice inoculated with B16 melanoma when treated with vinblastine and bleomycin (VB). Clinical responses have been observed in 28 of 137 patients (approximately 20%) treated with vinblastine (2), but bleomycin given by infusion has never been evaluated. In recent clinical trials in patients with metastatic melanoma, a response rate of 48.5% was achieved using bleomycin, CCNU, and vincristine (3), and a response rate of 45% was achieved combining DTIC with these three drugs (4). Results in these and other studies suggested that the VB combination might merit further examination and, therefore, the present study was initiated. During the course of study, the vinblastine dose employed proved highly myelosuppressive, prompting the use of a lower dose together with *cis*-dichlorodiammineplatinum(II) (cisplatin). This agent has been shown to produce objective responses in malignant melanoma when administered by iv (5) and intra-arterial (6) routes.

MATERIALS AND METHODS

Eligibility criteria included the following: histologic confirmation of incurable melanoma, performance status of < 3 ($> 25\%$ of time out of bed), measurable disease, BUN level of < 25 mg/100 ml, creatinine level of < 1.25 mg/100 ml, bilirubin level of < 2.0 mg/100 ml, platelet count of $\geq 100{,}000/mm^3$, and wbc count of $\geq 4000/mm^3$. All patients had completely recovered from the effects of previous chemotherapy and/or radiation therapy, had no evidence of infection, and had had no prior therapy with vinblastine, bleomycin, or cisplatin. Nine patients, including five objective responders, had been previously treated with DTIC (seven patients), methyl-CCNU (five), hydroxyurea (five), alkylating agents (three), or other drugs (two) (table 1). Eleven patients were treated with VB and three were treated with VB and cisplatin (VBD) (table 2). An adequate therapeutic trial was defined as a minimum of two courses. The maximum number of courses administered to any one patient was seven (mean, 2.4).

Responses were defined by Eastern Cooperative Oncology Group criteria as follows: *complete response*, the disappearance of all measurable lesions; *partial response*, a $> 50\%$ decrease from original measurements; and *progressive disease*, a $\geq 50\%$ increase over original measurements.

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[2]This study was performed in part by the Eastern Cooperative Oncology Group (*Mario Kuperminc*, Melanoma Committee Chairman) (pilot study EST P-B-678) and was supported in part by Public Health Service grants CA-07190 and CA-23776 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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TABLE 1.—Clinical features

Regimen	Sex M/F	Mean age	Mean duration of disease prior to therapy in mos (lymph nodes, subcutaneous, skin)	No. of patients with—												
				Performance status level				Sites of metastasis						Prior therapy		
				0	1–2	3	Unknown	Soft tissue	Liver	Bone	Lung	Bowel	CNS	Chemo-therapy	Immuno-therapy	None
VB	8/3	46	38 (5–93)	4	4	2	1	9	5	3	3	3	3	8	—	3
VBD	3/0	51	102 (16–228)	2	1	—	—	2	2	1	1	—	—	1	1	1
Totals	11/3	47	53 (5–228)	6	5	2	1	11	7	4	4	3	3	9	1	4

TABLE 2.—Drug schedules

Regimen	Dose	Schedule	Route
Vinblastine	8 mg/m^2	Days 1 and 2	Iv pulse dose
Bleomycin	20 units/m^2	Days 1–5	24-hr iv infusion
Vinblastine	6 mg/m^2	Days 1 and 2	Iv pulse dose
Bleomycin	15 mg/m^2	Days 1–5	24-hr iv infusion
Cisplatin	25 mg/m^2	Days 4 and 5	1-hr iv infusion with furosemide, mannitol, and vigorous hydration

Toxicity

Three of the 14 patients entered in the study were not fully evaluable for toxicity because of inadequate documentation or subjective toxicity (one patient), protocol violation (one), and too recent an entry in the study (one).

Patients experienced significant toxic effects with this regimen (table 3). Leukopenia was the major dose-limiting toxic effect. Three patients had leukopenia which was complicated by infection amenable to antibiotic therapy. Reduction of platelet count occurred to a lesser extent than reduction of wbc count. The platelet count never reached a nadir of $< 50{,}000/mm^3$. The nadir of suppression occurred between Days 4 and 7.

All patients experienced gastrointestinal toxic effects which were characterized by either nausea or vomiting or abdominal pain and constipation. The former began within a few hours of treatment, particularly with cisplatin, and usually disappeared within 4–6 hours. The onset of abdominal pain and constipation usually occurred between Days 2 and 7; in several patients these toxic effects continued for 2 weeks. This toxicity was almost always resolved by Day 28 and was attributed to the neurotoxicity of vinblastine.

A fall in the diffusion capacity for carbon monoxide (DCCO), as measured by pulmonary function tests, was noted in four patients. In three of these patients, the change was minimal and the patients were asymptomatic. When the pulmonary function tests were performed in these three patients their total doses of bleomycin were 150, 275, and 475 units. The fourth patient developed fever and shortness of breath after his seventh and last course of therapy (total, 584 units of bleomycin). A repeat pulmonary function test demonstrated an increase in restrictive disease and a further decrease in DCCO. A chest roentgenogram failed to reveal any infiltrate, but an increase in basilar interstitial markings was seen, probably due to atelectasis.

Significant and prolonged nephrotoxicity was seen in only one patient receiving total doses of 70 mg of vinblastine and 625 units of bleomycin (with no cisplatin) who had previously received bacillus Calmette-Guérin. During his fourth course of treatment his creatinine level rose to 1.9 mg/dl and his BUN level was 37 mg/dl. Further

TABLE 3.—Drug toxicity

	No. of patients				
	Degree of toxicity*				
Toxic effects	None	Mild	Moderate	Severe	Life-threatening
Wbc count	—	—	1	6	4
Platelet count	3	3	5	—	—
Nausea or vomiting	—	6	1	3	1
Abdominal pain	5	2	1	3	—
Constipation	5	1	4	1	—
Neurologic	8	—	2	1	—
Pulmonary	7	3	1	0	—
Renal	5	5	—	1	—
Stomatitis	6	1	3	1	—
Fever	3	2	3	3	—
Alopecia	2	—	3	6	—
Skin rash	10	—	1	—	—
Phlebitis	9	—	2	—	—

*Life-threatening: wbcs $< 1000/mm^3$ and platelets $< 25{,}000/mm^3$; severe: wbcs $< 2000/mm^3$ and platelets $< 50{,}000/mm^3$; moderate: wbcs $< 3000/mm^3$ and platelets $< 75{,}000/mm^3$; mild: wbcs $< 4000/mm^3$ and platelets $< 100{,}000/mm^3$. Severe: BUN > 50 mg/dl and creatinine > 5 mg/dl; moderate: BUN > 20 mg/dl and creatinine > 1.0 mg/dl.

TABLE 4.—Response data

Regimen	No. of patients		No. of responses*						Disease sites										Duration of response (mos)
									No. of responses in—					No. of relapses in—					
	Total	Evaluable	CR	PR	NC	P	TE	PV	Liver	Lung	Soft tissue	Bone	Bowel	CNS	Lung	Soft tissue	Bone		
VB	11	9	—	4	—	5	1	1	2	1	2	1	1	1	1	—	—		1,3+,5+,5
VBD	3	3	—	3	—	—	—	—	1	1	2	—	—	2	—	1	1		3+,3,8
Totals	14	12	—	7	—	5	1	1	3	2	4	1	1	3	1	1	1		1,3+,3+,3,5,5+,8

*CR = complete response; PR = partial response; P = progressive disease; NC = no change; TE = too early; PV = protocol violation.

evidence of progressive renal failure was noted 4 weeks later when his creatinine level was 2.1 mg/dl and his BUN level was 4.3 mg/dl. His creatinine and BUN levels continued to increase, reaching a peak of 7.5 and 144 mg/dl respectively.

Five other patients, two of whom were treated with cisplatin, showed minor transient elevations of BUN and creatinine levels ranging from 19 to 25 mg/dl and from 1.0 to 1.4 mg/dl respectively.

RESPONSE

Two of the 14 patients entered in the study are not fully evaluable: one because of protocol violation and the other because of inadequate trial.

Table 4 shows the response data. All three of the patients receiving the VBD regimen and four of the nine patients receiving the VB regimen responded. Thus, 58% of evaluable patients experienced objective remission of disease. The median duration of response was 3.0+ months for the entire group of patients.

Objective regression was seen in six patients in visceral sites with or without soft tissue metastases and in one patient with soft tissue metastases. Four of the patients who responded have experienced disease progression. One patient had progression of a previously known lung metastasis and the other three patients relapsed with new CNS disease (accompanied by new pulmonary nodules in one patient).

Two patients with progressive disease received only one course of chemotherapy. These were the only patients with a performance status of 3 at the time of entry in the study.

Five of nine patients with prior therapy and two of three without prior therapy responded.

DISCUSSION

This study was carried out in patients with far-advanced disease, most of whom had experienced prior chemotherapy. The regimen administered resulted in seven partial responses among 12 eligible patients. To date, the responding patients have a median survival time of 6.0+ months, with three patients still in remission. Nonresponders have a median survival time of only 2 months. Most patients experienced significant toxic effects. VB appears to have clinical effectiveness in the treatment of malignant melanoma, although remissions were short. This study may illustrate the principle that seemingly ineffective agents may have enhanced response rates when used in optimal schedules (ie, bleomycin infusion) or in combinations. Although the therapeutic contributions of cisplatin are not evaluable in this study, it may allow a decrease in otherwise intolerably toxic doses of VB while maintaining a high objective response rate. A further study with the VBD regimen is being undertaken in patients who have less-advanced disease, no prior chemotherapy, and a better initial performance status. In addition, because of the frequent occurrence of the CNS as a site of relapse, a program of prophylactic therapy for the CNS is being considered.

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Procarbazine, Vincristine, CCNU, and Cyclophosphamide (POCC) in the Treatment of Metastatic Malignant Melanoma[1,2]

Mark R. Green,* Robert O. Dillman, and Carol Horton[3,4]

SUMMARY

Twelve previously untreated patients with histologically documented, metastatic, malignant melanoma were treated with the combination of procarbazine, vincristine, CCNU, and cyclophosphamide, with an objective response rate of 33%. The median survival time was 10 months for responders versus 5 months for nonresponders. Bone marrow suppression was the major toxic effect. Further clinical trials are needed to better assess the utility of this combination in advanced melanoma.

[Cancer Treat Rep 64:139–142, 1980]

The efficacy of chemotherapy for metastatic malignant melanoma has been very disappointing (1,2). Of many single agents tested, only DTIC has been demonstrated to prolong survival in responders. Unfortunately, the overall response rate to DTIC is only about 20%, with a complete response rate of 5% (3). In spite of the theoretic advantages of combination chemotherapy, no combination has yet been shown to produce a significantly higher response rate than single-agent DTIC. Other agents do have response rates similar to those observed with DTIC: procarbazine (28%), vincristine (20%), CCNU (13%), and cyclophosphamide (22%) (2). These four agents form a reasonable combination (POCC) to investigate in the treatment of melanoma, not only because of their known single-agent activity in the disease, but also because (*a*) the POCC combination has been demonstrated to produce tolerable toxicity in the treatment of oat cell carcinoma (4), (*b*) the brain is a common site for melanoma metastases and three of these agents cross the blood-brain barrier and have been used in the treatment of CNS malignancies (5), and (*c*) the cyclophosphamide-vincristine and cyclophosphamide-vincristine-procarbazine combinations were reported to have produced responses in melanoma patients (three responses among ten patients and two among six patients respectively) in Australia (6), and CCNU-vincristine combination was shown to be superior to CCNU alone in a comparative study (20% versus 4% responses) by the Western Cancer Study Group (1).

MATERIALS AND METHODS

From January 1977 to May 1979, 12 patients with histologic documentation of melanoma with measurable, nonregional metastases were treated with POCC at four San Diego, CA, hospitals (Balboa Navy, Kaiser, University, and the San Diego Veterans' Administration). Requirements for entry in the study included absence of prior systemic chemotherapy, an estimated survival of > 6 weeks, and a wbc count of $> 3500/mm^3$ and a platelet count of $> 120{,}000/mm^3$. Cbc, platelet count, renal and hepatic blood chemistries, chest roentgenogram, ECG, and brain, bone, and liver scans were obtained at the time of entry.

The POCC regimen consisted of cyclophosphamide at a dose of 600 mg/m^2 iv on Days 1 and 8, vincristine at a dose of 1.4 mg/m^2 (maximum, 2 mg) iv on Days 1 and 8, CCNU at a dose of 60 mg/m^2 orally on Day 1 only, and procarbazine at a dose of 100 mg/m^2 orally on Days 2–15 of a 28-day cycle. Dose modifications were based on the wbc and platelet counts on Days 1 and 8 of each cycle. All patients who received two cycles of POCC were con-

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sidered evaluable, and all of the 12 patients entered completed at least two cycles.

Complete response was defined as a 100% regression of all measurable disease and the complete disappearance of all signs and symptoms attributable to tumor. Partial response (PR) was defined as a > 50% reduction in the sum of the products of two perpendicular diameters of all measured lesions and symptomatic benefit. Improvement was defined as a 25%–50% reduction in the sum of the products of perpendicular diameters of all measured lesions. Stable disease was defined as any improvement of a lesser degree or absence of change. Progressive disease was defined as the appearance of new metastases or a 50% increase in pre-existing lesions. Duration of response was defined as the date of chemotherapy initiation to the date of progression, relapse, or death. Survival was defined as the interval from the first date of POCC therapy to the date of death. The study was approved by the Human Subjects Committee of the University of California at San Diego, and written informed consent was obtained from all patients.

RESULTS

There were eight females and four males entered in the study. Patient characteristics are summarized in table 1. The average age was 56 years. No patient had received prior chemotherapy for his disease, but three patients had received prior immunotherapy, one had received regional limb perfusion with melphalan, and four had received radiation therapy (three to the brain only). Of the 12 patients, 11 had lung metastases, six had skin metastases, four had bone metastases, three had liver metastases, three had brain metastases, and three had lymph node metastases. Only three patients had a single site of disease identified. The diagnosis of melanoma had been made in eight patients within 3 years prior to entry in the study and in four patients within 6–22 years prior to entry. All patients had a Karnofsky status of ≥ 30% at the time of entry. Only two patients had a Karnofsky status of < 50%.

There were two CRs and two PRs (all females), with an overall response rate of 33%. Two other patients showed improvement. All of these six patients who demonstrated some disease regression did so prior to the second cycle of therapy. Survival curves for this small group reveal a median survival time for nonresponders of 5 months compared to 10 months for responders (CRs plus PRs). Two patients who have responded are still in the study after 3 and 7 months of treatment. The mean duration of response will be > 7 months.

Both females who achieved CRs had identifiable disease limited to one site and both had originally been diagnosed many years earlier (17 and 22 years). The other ten patients had been diagnosed an average of 3 years before entry in the study (median, 2 years). The six patients who showed a ≥ 25% improvement had been diagnosed an average of 10.2 years prior to the initiation of POCC compared to 1.5 years for the six patients who had stable disease or disease progression ($P < 0.05$ on a two-tailed test).

There were no instances of new brain metastases or disease progression of prior CNS disease in the absence of systemic progression of metastases during the study. One patient (No. 8) developed new brain lesions as her lung lesions were progressing and another patient (No. 5) had progression of her old brain lesions at a time of relapse of liver disease and progression of previously stable lung lesions.

Toxicity

POCC chemotherapy was reasonably well-tolerated (table 2). Ten patients had dose adjustments because of bone marrow toxicity and one because of vincristine toxicity. One of the ten patients with moderately severe leukopenia (wbc count, 1000–2000/mm^3) died of pneumococcal pneumonia and sepsis while leukopenic (No. 9). Another patient, who in the past had received immunosuppressive therapy with azothioprine for idiopathic thrombocytopenic purpura, had a nadir wbc count of < 500/mm^3. There were no bleeding complications among the thrombocytopenic patients. All of the patients entered the study with mild to moderate anemia and none had a hemoglobin level decrease to < 9.5–10.0 g/dl. Gastrointestinal toxicity was acceptable.

DISCUSSION

The preliminary results of this study suggest that POCC is a reasonably well-tolerated combination program for treating melanoma, with response rates similar to other regimens including BCNU-vincristine-DTIC (7–9) and bleomycin-vincristine-CCNU (10). The 17% CR rate in this small number of patients is remarkably high, but the limited extent of disease in the two patients with CR and their previous long survival time from the original diagnosis to POCC chemotherapy may limit the significance of this finding. Our findings are in keeping with the findings of other investigators who have noted increased response rates in females and in patients with soft tissue, nodal, and lung metastases. These results once again emphasize that the study population in any report concerning a small number of melanoma patients must be closely scrutinized because of the importance of such variables as sex, total duration of disease, prior therapy, and sites of metastases on response to therapy. Additional trials for treatment of metastatic melanoma with new single agents and a variety of combination

TABLE 1.—Clinical features and response to POCC chemotherapy for metastatic malignant melanoma

Patient No.	Hospital*	Age	Sex	Site of the original diagnosis	Yrs since original diagnosis	Metastatic sites at time of entry in the study	Response	Duration of response (mos)	Survival (mos)
1	K	66	F	Left ear	22	Lung	CR	13	16
2	U	72	F	Right ankle	17	Skin	CR	6+	—
3	U	63	F	Left ankle	2	Lung, skin, bone	PR	5½	9½
4	U	59	F	Right temple	3	Lung, nodal, brain	PR	2+	—
5	K	51	F	Left shoulder	11	Lung, liver, brain	Improvement	5	6
6	K	63	M	Right thumb	6	Lung, skin, liver, bone	Improvement	3	4½
7	U	29	F	Back	2	Lung, skin, other viscera†	Stable disease	2½	3
8	N	48	F	Back	2	Lung	Stable disease	4½	8
9	VA	51	M	Left wrist	1½	Lung, nodal, brain	Stable disease	2	2
10	VA	36	M	Scalp	1¼	Lung, skin, bone	Progressive disease	—	6½
11	U	61	M	Right great toe	¾	Lung, nodal skin, liver, bone	Progressive disease	—	5
12	U	77	F	Left heel	1½	Lung, skin	Progressive disease	—	4

*K = Kaiser, U = University, N = Balboa Navy, VA = San Diego Veterans' Administration.
†Not evaluated with liver, bone, or brain scan.

TABLE 2.—Toxic effects in 12 patients with melanoma treated with POCC

Toxic effect	No. of patients
Thrombocytopenia (cells/mm³)	
≥ 150,000	5
100,000–149,000	3
75,000– 99,000	0
25,000– 74,900	4
< 25,000	0
Leukopenia (cells/mm³)	
≥ 4000	2
3000–3999	3
2000–2999	2
1000–1999	4
< 1000	1
Hemoglobin decrease (g/dl)	
0–1.9	4
2.0–2.9	2
3.0–3.9	4
4.0	2
Nausea and vomiting	5
Neurologic	3

agents are in order. These trials should include further investigation into the utility of the POCC regimen in this disease.

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Combination Cytotoxic Chemotherapy With CCNU, Procarbazine, and Vincristine in Disseminated Cutaneous Malignant Melanoma: 3 Years' Followup [1,2]

J. Carmo-Pereira,* F. Oliveira Costa, Pedro Pimentel, and Elvira Henriques [3,4]

SUMMARY

Thirty patients with disseminated cutaneous malignant melanoma were treated with a three-drug cytotoxic chemotherapy program consisting of CCNU, procarbazine, and vincristine. Eighteen of 30 patients (60%) showed objective regressions, including ten complete responses (33%). Responders had cutaneous, nodal, and/or pulmonary involvement, but objective regressions were not achieved in patients with hepatic or brain metastases. The median response duration was 8 months and the median survival of the responders was 20 months compared to 5 months for nonresponders. These promising preliminary results require confirmation.

[Cancer Treat Rep 64:143–145, 1980]

The overall response rate in disseminated cutaneous malignant melanoma to single- or combination-agent cytotoxic chemotherapy has been persistently low (1–3), with responses being of brief duration and probably not contributing to an improved quality of life or prolongation of survival. DTIC has been found to be the most effective single agent in the treatment of this disease and many investigators now use this drug in combinations. In September 1976, we published the results of a trial on the treatment of this tumor using a combination of BCNU, DTIC, and vincristine, resulting in an objective response rate of 35% in 20 patients studied (4). Subsequent lack of availability of DTIC prompted us to try a new regimen comprising CCNU, procarbazine, and vincristine. The promising preliminary results are reported herein.

PATIENTS AND METHODS

Thirty consecutive patients (18 men and 12 women) with advanced measurable histologically proven cutaneous malignant melanoma, stages III and IV, were entered in this study between October 1, 1975, and February 29, 1979. The age range was 21–75 years with a median age of 55 years. Nine patients had received previous cytotoxic chemotherapy (hydroxyurea, DTIC, and BCNU, alone or in combination), but none had received any of the drugs included in the regimen being studied. Three had received immunotherapy with either bacillus Calmette-Guérin or dinitrochlorobenzene alone. None of the patients responded to these therapies, and the interval between withdrawal of prior treatment and the start of this protocol was always > 2 months, with the disease in progression.

Before starting cytotoxic chemotherapy patients were assessed for metastatic disease by physical, radiologic, and isotopic examination. All had a performance status of $\geq 50\%$ according to the Karnofsky scale (5). Baseline studies included biochemical tests of liver and renal function. Blood cell counts were determined at 2-week intervals and before each treatment course. The number of leukocytes had to be $\geq 4000/mm^3$ and the number of platelets had to be $\geq 90{,}000/mm^3$ for treatment to be given. Physical examination was repeated before each subsequent cycle of therapy. Appropriate radiographs and/or isotopic liver scans were done at 2-month intervals.

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[2]CCNU was initially supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]Clínica Oncológica VIII, Instituto Português de Oncologia, Francisco Gentil, Lisbon, Portugal.

[4]We thank Dr. R. D. Rubens, Medical Oncology Clinic, Guy's Hospital, London, England, for critically reviewing this manuscript and providing helpful comments.

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The regimen used was CCNU (150 mg/m^2 orally, maximum dose = 200 mg) on Day 1, procarbazine (100 mg/m^2 orally, maximum dose = 150 mg) on Days 1–10, and vincristine (1.4 mg/m^2 iv, maximum dose = 2 mg/dose) on Days 1 and 8, with a 4-week interval after each cycle. If myelotoxicity developed, ie, the leukocyte count fell to < 4000/mm^3 and/or the platelet count fell to ≤ 90,000/mm^3, the interval between the courses was increased until the number of leukocytes and platelets reached the values referred to above; myelotoxicity never caused the interval to exceed 8 weeks. If myelotoxicity did not develop, drugs were administered within 4 weeks.

The criteria used for objective evaluation of response were those recommended by the International Union Against Cancer (6).

The duration of response was determined not from the start of cytotoxic chemotherapy until disease progression, but from the date of remission occurrence until disease progression.

Survival was measured from the start of treatment to death or last followup and was analyzed by the life-table method.

RESULTS

Antitumor Effects

Thirty patients were studied. The overall response rate was 60% (18 of 30 patients). Ten complete responses (33%) and eight partial remissions (27%) were achieved. Twelve of the responses occurred in men (40%) and six in women (20%).

The median duration of remission was 8 months (range, 3–41+) and the median survival of the responders was 20 months (range, 4–42+) compared to 5 months (range, 3–17+) for nonresponders (fig 1) (P = 0.004).

The responses occurred in patients with both short and long disease-free intervals (table 1). The frequency of response was the same whether or not patients had received prior chemotherapy.

The majority of responders had cutaneous and/or nodal and pulmonary involvement (table 2). In patients with evident hepatic or brain metastases, objective regressions were not achieved with this schedule, as has been frequently reported by other investigators (7–9).

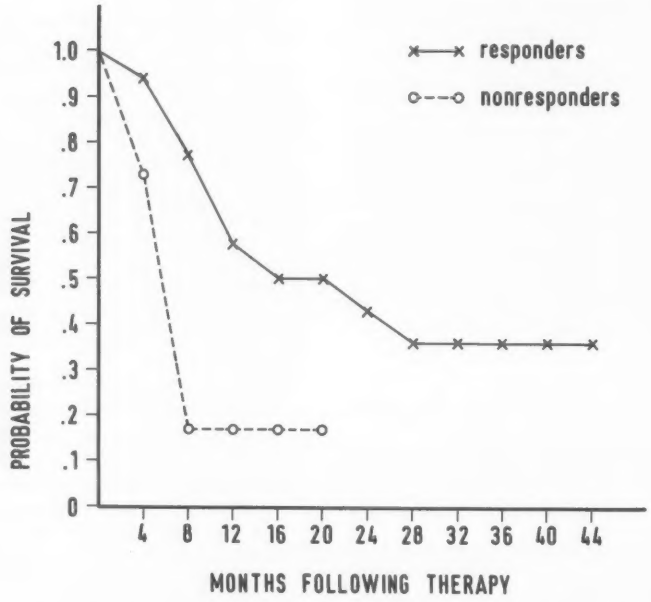

FIGURE 1.—Overall survival of responders (median, 20 mos) and nonresponders (median, 5 mos) (life-table method).

TABLE 1.—Response according to disease-free interval

Disease-free interval (yrs)	No. of patients treated	No. of responders
< 1	16	9 (56%)
1–5	12	9 (75%)
> 5	2	0
Total	30	18 (60%)

TABLE 2.—Response according to site of disease involvement

Site of disease involvement	No. of responders/ total No. with involvement
Skin and/or lymph nodes	14/20 (70%)
Visceral	
Lung	4/5 (80%)
Liver	0/3
Brain	0/2

Toxic Effects

The number of courses given to each patient varied from three to eight and the tolerance to treatment was generally good until the sixth to eighth cycle. Some patients who reached this number of courses refused to continue treatment because of general malaise or continuous nausea during the 10 days of procarbazine administration. In five patients persistent leukopenia developed, precluding further treatment.

The toxic effects observed are summarized in table 3. Alopecia and peripheral neuropathy did not occur and there were no drug-related deaths.

DISCUSSION

Stage III or IV disseminated cutaneous malignant melanoma remains a challenging disease for the chemotherapist. The results we have achieved, a 60% objective response rate (33% complete responses), are unexpectedly good, indicating that the regimen used here is particularly effective in the treatment of patients with this disease.

The responses always occurred rapidly, within 2–4 weeks of starting treatment. Responders survived significantly longer than nonresponders and the tolerance to the regimen was good.

TABLE 3.—Toxicity

	No. of patients with toxicity/ total No. treated
Hematologic	
Leukopenia (< 4000 cells/mm^3) Lowest leukocyte count observed: 800/mm^3	16/30 (53%)
Thrombocytopenia (< 100,000 cells/mm^3) Lowest platelet count observed: 50,000/mm^3	8/30 (27%)
Gastrointestinal	
Nausea and/or vomiting	28/30 (93%)
Diarrhea	0/30

These encouraging results require confirmation in a further series of patients with this heterogeneous disease.

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Phase II Study of VM-26 in Adult Malignancies [1,2]

Ellen Spremulli,* Joseph J. Schulz, Vincent J. Speckhart, and Galen L. Wampler [3]

Brief Reports and Preliminary Communications

In the last several years there has been a great deal of interest in the development of plant products for use in chemotherapy. One such compound is VM-26 (1–6).

MATERIALS AND METHODS

In 1976, the Virginia Oncology Group undertook a phase II study of VM-26 (supplied by the National Cancer Institute, Bethesda, MD) which was given weekly by iv infusion over 30 minutes. The dose was 100 mg/m^2 for patients with a wbc count of $> 4000/mm^3$ and a platelet count of $> 100,000/mm^3$. Half doses were given to patients whose wbc counts were between 3000 and 4000/mm^3 and/or whose platelet counts were between 75,000 and 100,000/mm^3. Treatment was withheld if blood cell counts were below these levels.

Eligible patients had histologic or cytologic confirmation of a malignancy which was incurable by surgical or radiotherapeutic measures. They were required to have failed conventional therapy and higher priority investigational treatment and to have had a 4-week rest from previous therapy prior to entry in the study.

Ninety-six patients with a median age of 55 years were entered in the study. Most patients (58.7%) had had extensive prior treatment, defined as three or more drugs or combinations of drugs or two or more drugs or combinations of drugs plus radiation therapy. Only 6.5% of patients had not received prior chemotherapy or hormonal therapy.

Twenty-nine of the 96 patients (30.2%) had an Eastern Cooperative Oncology Group performance status of 3 at the start of treatment. A performance status of 0 was recorded for 5.3% of the patients, while a performance status of 1, 2, and 4 was recorded for 23%, 28%, and 7.4% of the patients respectively. Five patients did not have their performance status recorded at the start of therapy. Forty-eight percent of the patients received between two and five doses of VM-26. Reasons for stopping treatment included disease progression documented by tumor measurement (23 patients [24%]); clinical progression defined as a $> 10\%$ weight loss, decreased performance status (point decrease of ≥ 2), or major changes in blood chemistries or hemoglobin (ten patients [10.5%]); severe side effects of medication (five patients [5.2%]); no improvement after 8 weeks of treatment (one patient [1.0%]); death (30 patients [31%]); disease progression after response (six patients [5.3%]); patient refusal of therapy or loss to followup (six patients [6.3%]); and other or unknown (15 patients [15.6%]).

Ten patients were excluded from the response analysis for the following reasons: death within 10 days of initial treatment (six patients), concurrent therapy (two), and insufficient data (two). Thirteen patients were excluded from toxicity evaluation due to inadequate followup data. Additional patients were excluded from evaluation of specific toxic effects when data were unavailable.

RESULTS

Toxicity

VM-26 was well-tolerated by the patients, although leukopenia was common. Thrombocytopenia was uncommon except in patients who had received nitrosourea therapy (*see* table 1). The incidence of marrow suppression seen is similar to that reported by others.

Six patients (6.3%) experienced nausea and/or vomiting; in two of these patients it was moderate and in four it was mild. One patient developed diarrhea which was attributed to the VM-26. Two patients developed alopecia, two had dizziness, and one had itching and erythema. Except for a few cases of marked marrow suppression seen in extensively treated patients, the only serious side effects were three cases of anaphylactoid reactions from which recovery was complete.

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[3]This study was done by the Virginia Oncology Group. The following members participated: *E. Spremulli* (University of Kentucky, Lexington); *J. J. Schulz* (James River Clinic, Newport News, VA); *V. J. Speckhart* (Medical Oncology Associates, Norfolk, VA); and *G. L. Wampler* (Medical College of Virginia, Richmond). Patients (No.) in this study were contributed by: J. J. Schulz (35), E. N. Spremulli (17), V. J. Speckhart (12), M. Kuperminc (10), R. L. Burger (6), G. L. Wampler (5), R. D. Diasio (3), W. Regelson (2), G. B. Rao (2), J. M. Meuller (1), W. K. Smith (1), D. M. Stith (1), and D. H. Riddick (1).

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TABLE 1.—Marrow suppression of VM-26

	No. of patients	% of patients— With leukopenia	% of patients— With thrombocytopenia	% of total patients
Total evaluable for marrow suppression	81	62.0	22.0	
Nadir wbc count (cells/mm³)				
3000–4000	14	28		17.3
2000–2999	20	40.0		25.0
1000–1999	12	24.0		14.8
< 1000	4	8.0		5.0
Nadir platelet count (cells/mm³)				
50,000–100,000	15		83	18.5
25,000–49,999	2		11	2.5
< 25,000	1		5.5	1.2

TABLE 2.—Classification and response of patients treated with VM-26

Tumor	No. of patients	No. of patients excluded from analysis	No. of patients with— Evaluable disease	Measurable disease	Evaluable response	Measurable response
Breast	23	3	11	9	4	1
Colon	20	3	3	14	0	2
Glioma	10	0	10	0	2	0

Tumor Response

Patients with both measurable and evaluable disease were entered in the study. Measurable disease was defined as disease which was measurable in two dimensions by ruler or calipers. A complete response was defined as the complete disappearance of all clinically detectable malignant disease without development of new malignant lesions lasting for at least 4 weeks. Partial response was defined as a $\geq$ 50% decrease in tumor size lasting for at least 4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease. Definite improvement in evaluable malignant disease was defined as an estimated improvement by > 50% which was agreed upon independently by two investigators. The response had to last at least 4 weeks and be documented by serial roentgenograms, scans, photographs, or abdominal girth measurements. Twenty-three patients with breast cancer, 20 with colorectal cancer, and ten with gliomas were treated, and their responses are detailed in table 2.

In patients with measurable disease, responses were seen in jejunal adenocarcinoma (one of one patient), colorectal carcinoma (two of 14), lymphoma (one of four), and small cell carcinoma of the lung (one of two). In patients with evaluable disease, partial responses were recorded in breast carcinoma (four of 11 patients), squamous cell carcinoma of the lung (one of two), malignant glioma (two of ten), and ovarian carcinoma (one of two). Our response rates are comparable to those reported in the literature (7–9).

DISCUSSION

We found VM-26 to be a drug with low activity but high acceptability in our extensively treated advanced cancer population. Marrow depression was the main toxic effect and was easily managed. In those patients who were able to continue receiving treatment for extended periods, there was no evidence of cumulative marrow toxicity.

We conclude that VM-26 may have some activity against a spectrum of adult solid tumors including malignant gliomas and carcinomas of the breast, colon, and ovary. Although only a few responses were seen in this study, it must be remembered that this patient population was very heavily pretreated. We found that patient acceptance was excellent and that toxicity was reversible.

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Randomized Trial of Chlorpromazine, Caffeine, and Methyl-CCNU in Disseminated Melanoma[1]

Brief Reports and Preliminary Communications

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SUMMARY

One hundred and fifty-seven evaluable patients with advanced metastatic malignant melanoma were randomly assigned to receive either methyl-CCNU (MeCCNU) (200 mg/m² orally every 6 weeks) (82 patients) or a combination of MeCCNU, chlorpromazine (50 mg/m² im), and caffeine (600 mg/m² sc) in the periumbilical area (75 patients). The response rate was 12% for the combination (three complete responses and six partial responses) and 11% for MeCCNU alone (two complete responses and seven partial responses). The median survival was 20 weeks and was the same for both treatments. The data support the hypothesis that caffeine and chlorpromazine do not enhance MeCCNU activity in malignant melanoma, unlike the marked enhancement seen for this drug combination in L1210 leukemia in mice.

[Cancer Treat Rep 64:151–153, 1980]

Chlorpromazine and caffeine markedly enhanced the antitumor effects of BCNU in murine L1210 leukemia (1,2). Using BCNU alone, there were 5% cures in this system. Addition of chlorpromazine and caffeine increased the cure rate to 70%. Optimal therapeutic results were obtained when chlorpromazine was given 6 hours before BCNU and caffeine was given immediately after BCNU.

Chlorpromazine increases the radiosensitivity of melanoma tissue, improves therapeutic response in murine melanoma, and moderately enhances the antitumor effect of chemotherapeutic agents in several experimental tumors (3–6). In one human melanoma trial, addition of chlorpromazine to BCNU, vincristine, and DTIC did not increase the response rate (7).

Caffeine's mechanism of action may relate to inhibition of DNA repair. Thus, caffeine or chloroquine treatment of cyclophosphamide-resistant hamster plasmacytomas restores sensitivity to cyclophosphamide, mechlorethamine, or irradiation (8). A randomized clinical trial evaluating caffeine, chloroquine, cyclophosphamide, and prednisone treatment in melphalan-resistant multiple myeloma patients failed, however, to demonstrate increased therapeutic benefit when DNA repair inhibitors were added (9).

A previous Eastern Cooperative Oncology Group melanoma study demonstrated that methyl-CCNU (MeCCNU) alone produced response rates similar to those produced by DTIC alone or MeCCNU plus DTIC with less drug-related toxicity (10). Based on these observations and the above experimental results, a prospective randomized trial of MeCCNU alone or with chlorpromazine and caffeine was initiated.

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, Chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following members participated: National Cancer Institute-Veterans' Administration Medical Oncology Branch, Washington, DC (*M. H. Cohen*); Sidney Farber Cancer Institute, Boston, MA (*D. Schoenfeld*, CA-23318); and Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*J. Wolter*, CA-10948). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); Baystate Medical Center, Springfield, MA (CA-20182); Brookdale Hospital Center, Brooklyn, NY; Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia, PA (CA-13611); University of Iowa, Iowa City; University of Florida, Jacksonville (CA-19819); Jefferson Medical College, Philadelphia, PA (CA-14215); University of Manitoba, Winnipeg, Canada; Mt. Sinai Medical Center, New York, NY (CA-17152); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Pennsylvania Hospital, Philadelphia (CA-13613); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); University of Montreal Affiliated Hospitals, Quebec, Canada (CA-21653); University of Rochester Cancer Center, NY (CA-11083); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, South Africa (CA-21692); SUNY-Stonybrook Veterans' Administration Hospital, Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Medical College of Virginia, Richmond (CA-10572); and Yale School of Medicine, New Haven, CT (CA-15485).

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PATIENTS AND METHODS

Between August 1974 and September 1975, 177 patients with biopsy-proven, surgically incurable melanoma were entered in a prospective randomized trial to compare the therapeutic effects of chlorpromazine, caffeine, and MeCCNU (CCM) versus MeCCNU alone. Twenty patients (14 randomized to CCM and six to MeCCNU) were nonevaluable due to ineligibility, cancellation, incorrect randomization, or loss to followup, leaving 157 evaluable patients (75 CCM patients and 82 MeCCNU patients).

Protocol eligibility required that the patients have normal hematologic function (wbcs $\geq$ 4000/mm^3 and platelets $\geq$ 150,000/mm^3) and renal function (BUN $\leq$ 25 mg/100 ml and/or creatinine $\leq$ 1.5 mg/100 ml). Full recovery from prior surgery, radiation therapy, and chemotherapy was required. Patients receiving prior nitrosourea therapy were excluded as were patients with second primary tumors, symptomatic cardiovascular disease, peptic ulcer disease, or a performance status of 4. Patients were required to have measurable disease and all gave informed consent.

Prior to randomization, patients were stratified by whether they had received prior chemotherapy and by performance status (0,1 versus 2,3). Treatment consisted of MeCCNU (200 mg/m^2 orally every 6 weeks) alone or with chlorpromazine and caffeine. Chlorpromazine (50 mg/m^2 im) was given 3–6 hours before MeCCNU and caffeine, and sodium benzoate (600 mg/m^2) was given sc in the periumbilical area (the caffeine concentration was 250 mg/ml, thus requiring a large injection volume) 15–30 minutes after MeCCNU. For patients randomized to MeCCNU alone no phenothiazine antiemetics were allowed.

Dose modifications of MeCCNU for hematologic toxic effects were based on nadir blood counts. Full drug doses were given if the nadir wbc count was $\geq$ 2500/mm^3 and the nadir platelet count was $\geq$ 100,000/mm^3. For nadir wbc counts between 1500 and 2499/mm^3 and nadir platelet counts between 50,000 and 100,000/mm^3, 80% of the calculated doses were given. Patients with nadir wbc and platelet counts below these levels received 60% of the MeCCNU dose.

Standard response criteria were used, with a complete response indicating complete disappearance of all measurable tumor and partial response indicating a $\geq$ 50% decrease in the sum of the products of perpendicular diameters of all measured lesions. A tumor response had to be maintained for two successive measurements at least 2 weeks apart. Progression required a 50% increase in the sum of the products of lesion diameters. Patients with progressive disease were subsequently treated at the discretion of the investigator but all patients were followed to death.

Standard statistical methods were used to compare patient response, toxicity, and survival. Costanza et al (10) identified prognostic variables of response and survival using the logistic and life-table regression models (11,12). Analysis of results by these methods gave results similar to those produced by simple treatment comparisons. To simplify this presentation the models were not used.

RESULTS

Table 1 compares the characteristics of the patients receiving CCM versus those receiving MeCCNU alone. The former group had somewhat more nonambulatory patients and a higher percentage of patients with prior chemotherapy. Brain and/or liver metastases were present in approximately 40% of the patients in each group. Patients with skin and/or lymph node metastases comprised only 32% of each group.

Response rates are indicated in table 2. The objective response rate for the 75 patients receiving CCM was 12% versus 11% for the 82 patients receiving MeCCNU. Response durations for the three complete responders to CCM were 84, 112, and 1034+ days versus 175 and 181 days for the two complete responders to MeCCNU. Response durations for the partial responders to CCM and MeCCNU were 14, 23, 48, 84+, 97, and 175+ days and 12, 38, 92, 114, 121, 122, and 1025 days respectively. There was no significant difference in response duration for the two treatments. The overall median response duration was 97 days. The median time to response was 12 weeks for CCM and 6 weeks for MeCCNU. This difference was not significant.

Ambulatory patients receiving CCM survived longer than those receiving MeCCNU (P = 0.1, two-sided).

TABLE 1.—Patient characteristics

Parameter	% of evaluable patients	
	CCM (75 patients)	MeCCNU (82 patients)
Performance status		
0,1	73	83
2,3	27	17
Previous surgery	96	98
Previous radiation	5	11
Previous immunotherapy	15	17
Previous chemotherapy	12	5
Metastatic sites		
Brain	13	15
Liver	29	26
Lung	39	45
Bone	23	13
Nodes	29	39
Soft tissue	31	33

TABLE 2.—Treatment response

Regimen	No. of responses*				
	CR	PR	NC	Progression	NE
CCM	3(4%)	6(8%)	5	56	5
MeCCNU	2(2%)	7(9%)	5	63	5

*CR = complete response, PR = partial response, NC = no change, NE = not evaluable.

Median survival for ambulatory CCM-treated patients was 27 weeks versus 20 weeks for comparable MeCCNU-treated patients. For nonambulatory patients median survival was 8 weeks for CCM treatment versus 13 weeks for MeCCNU treatment. The median survival for responders, measured from the start of response, was 35 weeks versus 19 weeks for nonresponders ($P = 0.01$).

Toxicity

Aside from one case of severe anxiety noted with the CCM treatment, the only significant toxic effects were vomiting and myelosuppression. CCM was associated with significantly less vomiting than MeCCNU ($P = 0.035$). Fifty-six percent of CCM-treated patients had no vomiting versus 42% of MeCCNU-treated patients. There was no significant difference in hematologic toxic effects. Seventy-one percent of patients receiving CCM had either no hematologic toxic effects (51%) or mild toxic effects (20%) versus 45% and 17% for no or mild hematologic toxic effects for MeCCNU alone. There were no treatment-related deaths in either group.

DISCUSSION

This study failed to demonstrate a therapeutic advantage for the CCM combination as compared to MeCCNU alone. An explanation for the disparate therapeutic results observed in murine test systems and in human melanoma presumably relates to tumor sensitivity to cytotoxic therapy. L1210 leukemia, even when advanced, is highly sensitive to nitrosoureas as are most murine melanomas (2,13). Human melanoma, as evidenced by results in this trial, is considerably less sensitive. In addition, response rates and survival in clinical trials are also negatively influenced by pretreatment prognostic factors such as poor performance status, prior chemotherapy, and the presence of brain and liver metastases (10). Over 40% of patients entering this trial had brain and/or liver metastases, 22% had poor performance status, and 8% had prior chemotherapy. For good performance status patients there was a trend toward improved survival in CCM patients but results were not statistically significant.

The addition of chlorpromazine and caffeine to MeCCNU treatment ameliorated nausea and vomiting and did not increase hematologic toxicity. Thus, it might be reasonable to test this drug combination in more responsive human tumors. The relatively mild hematologic toxicity observed in both treatment groups suggests that higher doses of MeCCNU might also have been used.

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Phenytoin-Misonidazole: Possible Metabolic Interaction [1]

Brief Reports and Preliminary Communications

Diamon Gangji, James G. Schwade, and John M. Strong [2,*]

SUMMARY

Following iv administration of misonidazole, evidence of decreased plasma half-life (3.8 hours versus 9.1 hours ± 0.8 [SD] in eight other patients studied) and increased metabolism was observed in a patient receiving continuous phenytoin therapy.

[Cancer Treat Rep 64:155–156, 1980]

The potential clinical usefulness of misonidazole, a hypoxic cell radiosensitizer, is being investigated at a number of centers. Two major dose-limiting side effects have been reported: gastrointestinal disturbances and neurotoxicity (both peripheral and central nervous system [CNS]) (1,2). Peripheral neurotoxicity, which appears more frequently, has been suggested by some authors to be related to the dose and length of exposure (2). The frequency and dose relationship of CNS toxicity are less clear. It is of interest that neurotoxicity associated with misonidazole has been reported with variable frequency by different investigators. This has also been the case for the related radiosensitizer, metronidazole, a 5-nitroimidazole, which is also being investigated. Urtasun et al (3) did not report any significant neurotoxicity in a group of patients with brain tumors treated with high doses of metronidazole, whereas Frytak et al (4,5), using metronidazole in lower doses in patients with colorectal carcinoma, encountered severe CNS toxicity.

Our observations in a patient receiving continuous phenytoin therapy while also receiving misonidazole during a phase I trial of an iv formulation of misonidazole might shed some light on this difference in toxicity and have important clinical implications.

MATERIAL AND METHODS

To date, kinetic studies after iv administration of misonidazole have been conducted in eight patients at the Radiation Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute. None had been previously treated with nitroimidazole or related compounds. All had normal liver and renal function. Serial blood and urine samples were collected over a 24-hour period. The misonidazole iv formulation was supplied in 500-mg vials by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Patients received total doses ranging from 0.5 to 4 g. The drug and its desmethylated metabolite, desmethylmisonidazole, were assayed in blood and urine samples using a modification of the gas chromatographic method reported by Flockhart et al (6).

RESULTS AND DISCUSSION

The plasma half-life in seven of these patients after iv administration was 9.1 hours ± 0.8 (SD) (range, 8–9.5 hours). In contrast, the eighth patient, receiving continuous phenytoin therapy, exhibited a much shorter half-life (3.8 hours) (table 1). Peak plasma levels were similar in both groups. In an additional patient receiving phenytoin (concomitantly with dexamethasone) we studied the misonidazole plasma half-life after oral administration. The misonidazole plasma half-life in this patient was 6.5 hours, shorter than the usually reported values of 12–14 hours (1,2,6,7). Although one of the patients was also receiving dexamethasone, based on recent observations in the rat it appears that it is phenytoin and not dexamethasone which modifies the rate of misonidazole disappearance from the plasma (8). A suggestion of increased metabolism in the patient receiving phenytoin while also receiving iv misonidazole is found in the comparison of the ratio of the metabolite, desmethylmisonidazole, to the parent compound, misonidazole, excreted in the urine over a 24-hour period: 1.46 ± 0.60 (SD) for

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TABLE 1.—Pharmacokinetic results following iv administration of misonidazole

	Control group (±SD)	Patient receiving phenytoin
Plasma half-life	9.1 ± 0.8	3.8
Ratio of metabolite to drug excreted in urine over a 24-hr period	1.46 ± 0.6	7.95
Renal clearance of misonidazole (ml/min)	3.4 ± 3	2.7
Plasma clearance of misonidazole (ml/min)	42.2 ± 13	125

the control group and 7.95 for the patient receiving phenytoin. Increased metabolism is also indicated in a comparison of the renal and plasma clearances of the parent compound between the control group and the patient receiving phenytoin (table 1).

These data suggest an increased metabolism of misonidazole in patients taking phenytoin. Animal and human data indicate that liver metabolism plays a major role in misonidazole elimination (6,9). Therefore, the most likely rationale for this interaction might be the well-known effects of induction of microsomal liver enzyme by phenytoin (10). It is unlikely that protein binding competition plays any role, since misonidazole and related compounds do not bind to plasma protein (7).

This finding may explain the discrepancy of neurotoxicity reported by various investigators. For example, Weinstein et al (11), Walker and Strike (12), and T. L. Phillips (personal communication) have observed in a population of brain tumor patients a significantly lesser degree of neurotoxicity in those receiving oral misonidazole. This patient population is more likely to be treated with phenytoin.

The difference in the frequency of reports of neurotoxicity from Urtasun et al (3) and Frytak et al (4,5) may result from the different patient populations studied. Urtasun's brain tumor patients were taking phenytoin and dexamethasone, and, in addition, generally had normal liver function. On the other hand, Frytak's patients had advanced colorectal disease, were less likely to be receiving phenytoin or comparable drugs, and, in addition, may have had a higher incidence of liver metastasis with subsequent liver dysfunction.

It is possible that less neurotoxicity might be encountered in patients receiving phenytoin since it increases drug metabolism resulting in a shorter tissue exposure to misonidazole.

Further investigation is under way to confirm these preliminary findings. We suggest that clinical reports of misonidazole treatment should include additional information regarding both liver dysfunction and concomitant drug therapy.

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Chemoimmunotherapy for Metastatic Breast Cancer With 5-Fluorouracil, Adriamycin, Cyclophosphamide, Methotrexate, L-Asparaginase, *Corynebacterium parvum*, and *Pseudomonas* Vaccine[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Moderate doses of methotrexate and L-asparaginase were added to standard doses of 5-fluorouracil, Adriamycin, and cyclophosphamide in an attempt to improve the overall response rate and survival following chemotherapy. In addition, nonspecific immunotherapy with either *Corynebacterium parvum* or *Pseudomonas* vaccine was integrated into this prospective randomized clinical trial. The overall toxicity (degree of granulocytopenia and thrombocytopenia, length of myelosuppression, and incidence of myelosuppression-related infection and infectious deaths) increased considerably and led to the termination of patient accrual. The incidence of stomatitis and diarrhea was also increased with the addition of methotrexate and L-asparaginase, and apparently was potentiated by the addition of *Pseudomonas* vaccine to this five-drug combination.

[Cancer Treat Rep 64:157–159, 1980]

We designed a prospective randomized trial to determine (*a*) if the addition of L-asparaginase and methotrexate (1) to 5-fluorouracil, Adriamycin, and cyclophosphamide (FAC) (2) improved response rate and survival without additional toxicity and (*b*) if nonspecific immunotherapy with *Corynebacterium parvum* (CP) or *Pseudomonas* vaccine (PV) improved the therapeutic efficacy of combination chemotherapy for breast cancer.

The toxicity observed with the program was considerably greater than expected and the study was closed for patient accrual. We report here on the toxicity data and preliminary therapeutic results of our study.

MATERIALS AND METHODS

Patients with metastatic breast cancer and measurable lesions were admitted to the study. An informed consent was obtained from each patient.

Patients were stratified according to menopausal status, disease-free interval, number of organ sites involved, and dominant site of metastasis and were randomized to one of four treatment arms: *treatment A*—FAC alone, as previously described (2); *treatment B*—FAC and CP (2 mg/m^2 iv on Days 9 and 16 of each course); *treatment C*—FAC, L-asparaginase (10,000 units/m^2 im on Day 1), and methotrexate (120 mg/m^2 iv on Day 10); or *treatment D*—same as treatment C and PV (0.5 mg/m^2 sc on Days 7, 12, and 17 of each course). The criteria for dose modification, pretreatment and on-study evaluation, definition of response, and survival used in this study have been previously described (2).

Four months after starting this protocol, the supply of CP was exhausted. The accrual of patients for treatments C and D was stopped 6 months after the initiation of the study because of toxicity.

RESULTS

Seventy patients were entered in the trial and randomized to one of the four treatment arms as shown in table 1. Response rates and evaluability are also shown in table 1.

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Toxicity

Nonhematologic toxicity is shown in table 2. The incidence of stomatitis was considerably higher with the inclusion of methotrexate. The toxicity related to the administration of PV was considerable. All patients experienced pain and tenderness at the injection site, fever and chills, and malaise. The incidence and severity of side effects were higher in this present study than in our phase I trial (4). Side effects related to PV at this dose and schedule were unaccepatable for all but two patients, and resulted in discontinuation or significant dose reduction of the agent. Although none of the patients treated with FAC refused further therapy, only 40% of the patients receiving treatment C and 12% of the patients receiving treatment D remained in the study after 6 months.

Hematologic toxicity is described in table 3. Treatments A and B produced identical myelosuppression. Treatments C and D induced considerably more granulocytopenia and thrombocytopenia with each course of therapy and major dose reductions were required. After the first course of treatment, > 50% of the patients in the treatment C group and > 40% of the patients in the treatment D group had their dose of chemotherapy reduced by 20%. This is in sharp contrast with treatment A and with our previous experience with FAC. The onset of myelosuppression was early and was usually severe by Day 8, even though methotrexate was not administered until Day 10. In most cases, Day-8 5-fluorouracil was omitted for treatments C and D. The length of myelosuppression was prolonged and the interval between cycles increased to 28–35 days in most patients. There was a higher incidence of infection related to neutropenia. Among 37 patients entered in treatment arms A and B, only one developed a severe infection (septicemia) and only four developed fevers of unknown origin. In contrast, 13 of 33 patients in treatment arms C and D developed severe infections and four of these patients died from infection while experiencing neutropenia. Drug-related deaths were more frequent than in our previous studies (3).

DISCUSSION

The results of this trial show that the addition of moderately high doses of methotrexate and L-asparaginase to FAC combination chemotherapy significantly increased the incidence and severity of toxicity of the program. The addition of PV at the dose and schedule described in this protocol also caused a high incidence of side effects and toxicity in the majority of patients. While virtually no toxicity was observed with the L-asparaginase-methotrexate phase II program at 120 mg/m^2 of methotrexate, the two-drug combination appeared to add significant myelosuppressive and other toxicity to FAC.

TABLE 1.—Response

	Treatment—			
	A	B	C	D
Total No. of patients entered	15	22	12	20
No. of patients evaluable	12	14	10	16
Complete response	3 (25%)	1 (7%)	0	3 (19%)
Partial response	4 (33%)	6 (43%)	5 (50%)	11 (68.8%)
Stable disease	4	6	5	1
Progression	1	1	0	1

TABLE 2.—Nonhematologic toxicity

Toxic effect				
% of patients with—				
Nausea + vomiting	81	85	90	75
Stomatitis	18	10	40	90
Diarrhea	0	0	30	0
Alopecia	100	100	100	90
Infection, septicemia	7	0	31	25
Fever of unknown origin + neutropenia	20	4	14	35
No. of drug-related deaths	0	0	2	2

TABLE 3.—Hematologic toxicity

	Course No.	
	1	3
	Treatments A and B	
Lowest counts (cells × 10^3/mm^3)		
Wbcs	2.7	3.1
Absolute granulocytes	0.5*	1.0
(range)	1.1–1.5	0.6–1.6
Platelets	187*	160
(range)	120–405	89–340
Median % of dose administered	100	100
	Treatment C	
Lowest counts (cells × 10^3/mm^3)		
Wbcs	1.0	1.8
Absolute granulocytes	0.3	0.8
(range)	0–1.7	0.1–1.7
Platelets	117	120
(range)	37–228	42–277
Median % of dose administered	100	80
	Treatment D	
Lowest counts (cells × 10^3/mm^3)		
Wbcs	1.5	1.3
Absolute granulocytes	0.8	0.2
(range)	0–3.3	0–0.6
Platelets	130	140
(range)	15–275	38–190
Median % of dose administered	100	80

* Median.

Methotrexate is a valuable and effective agent in the treatment of breast cancer and its addition to FAC chemotherapy could improve the therapeutic ratio, provided this could be achieved without added toxicity. In this context, the addition of high-dose methotrexate with citrovorum factor rescue might be a more appropriate strategy (5). Similarly, while PV was not found to produce stomatitis in our phase I study (4), it appeared to potentiate the occurrence of mucositis in the FAC plus methotrexate program.

It is known that chemotherapeutic agents potentiate the toxicity of endotoxins in animal systems (6). There is a definite schedule-dependence, but potentiation is only observed with certain sequences of administration. A similar interaction may have occurred in this trial.

While the development of new treatment programs is necessary to improve the therapeutic results in metastatic breast cancer, caution should be exercised in the design of these studies and ample safety margin should be left for unexpected toxic manifestations.

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Phase II Study of 5-Fluorouracil and Adriamycin in Transitional Cell Carcinoma of the Urinary Tract[1,2]

Brief Reports and Preliminary Communications

Silvana Martino, Bohumil Samal, and Muhyi Al-Sarraf[3,*]

SUMMARY

A prospective study was carried out to determine the effectiveness and tolerance of the combination of 5-fluorouracil (600 mg/m²) and Adriamycin (50 mg/m²) given iv every 3 weeks to patients with disseminated transitional cell carcinoma of the urinary tract. Twenty-one of 23 patients entered in the study were evaluable for both response and toxicity. Two patients had complete responses and six had partial responses (≥ 50% reduction), yielding an overall response rate (complete plus partial) of 38%. Leukopenia (72.7%) and thrombocytopenia (54.5%) were common. Toxicity was life-threatening in one patient. Other common side effects were alopecia, nausea and vomiting, and a generalized feeling of weakness. Responders had a median survival time of 29 weeks compared to a median survival time of 9 weeks for nonresponders.

[Cancer Treat Rep 64:161–163, 1980]

Many cytotoxic agents have been used in the treatment of patients with advanced transitional cell carcinoma of the urinary bladder. Adriamycin and 5-fluorouracil (5-FU) are among the promising agents used in the treatment of these patients.

Adriamycin is the most thoroughly evaluated single agent, and has shown definite clinical activity in bladder cancer. The overall objective response rate was 23% (range, 0–57%) in a collected series of 235 patients (1–10). More recent reports showed lower response rates to Adriamycin. Yagoda et al (11) reported a 14% response rate with five of 35 adequately treated patients and Weinstein and Schmidt (12) reported a response rate of 5% with one of 19 patients experiencing objective remission. Soloway (13,14) has reported that Adriamycin is an effective single agent in primary murine bladder cancer.

5-FU has been found to be active against metastatic transitional cell cancer of the bladder. An overall response rate of 35% (range, 0–75%; 26 of 74 patients) has been reported. However, most of the studies contain a relatively small number of patients (3).

A prospective trial with the combination of Adriamycin and 5-FU was performed at our institution to evaluate the efficacy of this combination.

METHODS AND MATERIALS

From June 1976 to October 1977, 21 patients with transitional cell carcinoma of the bladder (one of the renal pelvis and one with transitional cell carcinoma arising in the urethra) were entered in the study. 5-FU at a dose of 600 mg/m² iv bolus and Adriamycin at a dose of 50 mg/m² iv bolus were given at 3-week intervals. Patients with prior extensive irradiation, or those judged to have received prior extensive doses of myelosuppressive chemotherapy, received reduced doses of 500 mg/m² of 5-FU and 40 mg/m² of Adriamycin.

Admission in the study required a histologically proven diagnosis of transitional cell carcinoma, advanced local disease, or disease metastatic to distant sites.

There were 17 males and six females with a mean age of 65 years (range, 44–83). Seven patients had advanced disease limited to pelvic structures, and the remaining 16 had distant metastases: cervical, axillary, and left supraclavicular lymph nodes, lungs, pericardium, subcutaneous nodules, bone, and liver.

None of the patients had received previous treatment with systemic chemotherapy. Sixteen patients had had previous radiation to the pelvic area.

RESULTS

For this study, a complete response (CR) was defined as the disappearance of all clinical evidence of tumor, and a partial response (PR) was defined as a ≥ 50% decrease in the sum of the products of the diameter of measurable lesions. One patient was lost to followup after the first

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course of therapy. One patient died 12 days after the first course of therapy and was evaluated for toxicity only. Two of our patients had CRs. A 69-year-old black male, with a left supraclavicular lymph node as the only evidence of distant metastatic disease, had a total clinical disappearance of his node following five courses of treatment. Cystoscopy also failed to demonstrate any disease. The node re-occurred 4 months thereafter while he was receiving therapy. The second patient who achieved a clinical CR was a 73-year-old white female with metastatic disease to the right lung, palpable masses in the right inguinal area lateral to a surgical scar, and a palpable rectal mass. All lesions could no longer be identified after two courses of therapy. The patient died from a pulmonary embolism shortly after she achieved a CR, but no autopsy was obtained to substantiate her CR.

An additional six patients achieved PRs, with an overall response rate (CRs plus PRs) of 38% (eight of 21 patients). The following lesions were measured and responded to the combination of 5-FU and Adriamycin: lung nodules (three patients), lymph nodes (two: one left supraclavicular and one mediastinum), pelvic mass (two), and recurrent bladder cancer (one who had cystoscopy every 2–3 months). The median duration of objective response was 8 weeks (range, 2–30). The median duration to achieve CR or PR was 10.5 weeks.

A comparison of performance status at the start of therapy demonstrated that responders had a median Karnofsky performance rating of 80% (range, 60%–100%) and nonresponders had a median performance rating of 60% (range, 35%–85%). This difference was statistically significant, $P = 0.027$. The median survival time of patients from the start of therapy to death was 29 weeks for responders and 9 weeks for nonresponders; however, this difference was not statistically significant ($P = 0.25$).

The main side effect was leukopenia (wbc count < 4000/mm^3) which occurred in 16 of 22 patients. Twelve patients had severe leukopenia (wbc count < 1999/mm^3), three had moderate leukopenia (wbc count, 2000–2999/mm^3), and one had mild leukopenia (wbc count, 3000–3999/mm^3). Thrombocytopenia was encountered in 12 of 22 patients. Of these 12 patients, six had severe thrombocytopenia (platelet count < 50,000/mm^3) and six had mild thrombocytopenia (platelet count, 50,000–99,999/mm^3). One patient became severely myelosuppressed and died with sepsis 12 days after the first course of therapy. In the 16 patients who had had prior radiotherapy, the lowest median wbc count observed with 5-FU and Adriamycin was 1600/mm^3 (range, 1000–9500/mm^3) and the lowest median platelet count was 90,000/mm^3 (range, 3000–280,000/mm^3). In the eight patients without prior radiotherapy, the lowest median wbc count observed with 5-FU and Adriamycin was 4600/mm^3 (range, 1300–8300/mm^3) and the lowest median platelet count was 187,000/mm^3 (range, 21,000–276,000/mm^3). The median lowest wbc and platelet counts were 2300 and 94,000/mm^3, respectively, for responders and 1800 and 196,000/mm^3, respectively, for nonresponders.

Other frequent side effects were alopecia, nausea and vomiting, and a feeling of weakness after injection of the drugs. Two patients developed mild stomatitis. No cardiac toxicity occurred.

DISCUSSION

Various systemic therapeutic agents have been used for advanced transitional cell carcinoma of the urothelium. Two of the most studied drugs are Adriamycin and 5-FU.

The range of reported responses (CRs plus PRs) to systemic Adriamycin has been considerable. Tan et al (9) and Odujinrin et al (8) each reported no responses in a small group of patients. Middleman et al (6) and Frei et al (5) reported response rates of 57% and 55%, respectively. Similarly, the reported response rates to systemic 5-FU range from 0 to 75% (3).

Combining these two agents resulted in a response rate of 38% in eight of 21 patients with either locally advanced or metastatic transitional cell carcinoma (1,15). Our results are comparable to those published by Cross et al (16) who reported a 35% response rate in 20 patients with advanced lesions. Similarly, the European Organization for Research on Treatment of Cancer Urological Group B (17) reported a 40% response rate in 52 patients which included some of Dr. Cross' patients.

In our study, patients with a higher Karnofsky performance rating at the start of therapy had a higher response, and those who responded to this regimen experienced a statistically significant increased survival time. Toxicity was life-threatening in one patient. The majority of our patients experienced moderate myelosuppression. No cardiac toxicity occurred.

Recently, Williams et al (18) reported an increased objective response rate of 62.5% (ten of 16 patients) in bladder cancer with the addition of *cis*-dichlorodiammineplatinum(II) to the 5-FU-Adriamycin combination.

The 5-FU-Adriamycin combination should be tried in patients with poor renal function who may not tolerate *cis*-dichlorodiammineplatinum(II), and certainly the three-drug combination should be further investigated in a phase III trial.

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Methotrexate and 7-Hydroxymethotrexate: Serum Level Monitoring by High-Performance Liquid Chromatography [1,2]

Brief Reports and Preliminary Communications

Claver Canfell[3,4] and Wolfgang Sadée[3,5,*]

SUMMARY

We have developed a reverse-phase high-performance liquid chromatographic assay with ultraviolet detection at 303 nm for methotrexate (MTX) and 7-hydroxymethotrexate (7-OH-MTX) in serum, plasma, and cerebrospinal fluid. The procedure involves the concentration of MTX and 7-OH-MTX in the aqueous phase by an *n*-butanol-ether extraction, resulting in a sensitivity of 10–20 nM in serum. Serum levels of MTX correlated well with those obtained by a competitive dihydrofolate reductase binding assay (r = 0.989). The clinical utility of the assay was demonstrated by measuring MTX and 7-OH-MTX serum levels in 42 patient samples.

[Cancer Treat Rep 64:165–169, 1980]

Therapeutic monitoring of serum methotrexate (MTX) levels has been introduced as a guide to adjust citrovorum factor rescue treatment after high-dose MTX infusions (1,2). A metabolite of MTX, 7-hydroxymethotrexate (7-OH-MTX), may contribute to the renal toxicity of MTX (3–5). The analytic monitoring techniques include high-performance liquid chromatographic procedures with ultraviolet (LC-UV) or fluorescence detection (4–7); however, none of the available analytic assays is capable of measuring serum levels of both MTX and 7-OH-MTX with the requisite sensitivity (10 nM) for clinical serum samples. We report an LC-UV assay for both MTX and 7-OH-MTX which is simple, sufficiently sensitive, and specific.

MATERIALS AND METHODS

Chemicals.—MTX (lot No. J-5192, Lederle Laboratories, Pearl River, NY) and 3′,5′-dichloromethotrexate were supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The MTX sample was 84% ± 2% pure anhydrous MTX (assayed by the Stanford Research Institute, Menlo Park, CA); this was confirmed by the UV absorptivity of a stock solution (10 mg of pure MTX/100 ml of water), assuming a molar absorptivity of MTX of 23,000 (8). Citrovorum factor was obtained from Lederle Laboratories and 4-amino-4-deoxy-N^{10}-methylpteroic acid was obtained from Dr. A. Rosowsky, Sidney Farber Cancer Institute, Boston, MA. A sample of 7-OH-MTX was obtained from Dr. David Johns, Division of Cancer Treatment, National Cancer Institute, and its purity was determined to be 77% by LC-UV analysis using peak area integration and assuming a molar absorptivity of 19,500 at 303 nm in the LC eluent (8). All other reagents were of analytic grade.

Apparatus.—LC analysis was performed using a model 6000A pump, a U6K injector, and a μBondapak/phenyl reverse-phase column (25 cm × 3.9 mm internal diameter) (all from Waters Associates, Milford, MA). Column eluents were monitored at 303 nm using a variable wavelength detector (Schoeffel Instruments Corp, Westwood, NJ). The mobile phase consisted of 0.15 M sodium acetate buffer (pH 4.6):acetonitrile (89:11) at a flow rate of 2 ml/minute and at room temperature (approximately 23°C).

Analytic procedure (for therapeutic MTX level monitoring).—Serum (plasma or cerebrospinal fluid, 1 ml) and 1 ml of acetonitrile were vigorously agitated for 10 seconds and centrifuged at 2000 × *g* for 10 minutes. The entire supernatant (~ 1.6 ml) was transferred by pipet into a nipple-bottomed screw-capped glass tube containing 5.5 ml of anhydrous ethyl ether and 2.9 ml of *n*-butanol. The glass tubes were shaken for 2 minutes and centrifuged at 2000 × *g* for 5 minutes, and the upper (organic) layer was discarded by aspirating with a trans-

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fer pipet using a vacuum. The remaining aqueous layer (< 100 μl) containing MTX and 7-OH-MTX was re-extracted with 2.5 ml of ether to remove the *n*-butanol contained in the aqueous phase. This entire aqueous concentrate was centrifuged in an Eppendorf microcentrifuge at 10,000 × *g* for 2 minutes prior to injection onto the column. MTX concentrations were determined by peak height or peak area comparisons (giving equivalent results) with a standard curve, prepared daily, using 0.11, 1.1, and 11 μM MTX in 1 ml of drug-free serum. Serum samples of up to 2 ml (with a twofold scale-up of extraction volumes) may be processed for maximum sensitivity. Since daily standard curves were prepared for MTX only, serum 7-OH-MTX concentrations were determined by multiplying the measured serum MTX concentrations by the LC peak area ratio (7-OH-MTX/MTX) as well as by a conversion factor (F = 1.18) which reflected the lower absorptivity of 7-OH-MTX at 303 nm.

Comparison assay.—Patient sera were analyzed in the Clinical Pharmacokinetics Laboratory, University of California at San Francisco, by the competitive dihydrofolate reductase binding assay of Myers et al (9), observing standard quality control procedures.

RESULTS AND DISCUSSION

The sensitivity of the LC-UV analysis of pure MTX was of the order of 10 pmols injected onto the column. The newly developed extraction procedure served to concentrate and purify the MTX and 7-OH-MTX in serum samples, thereby providing an assay sensitivity with adequate precision of 10–20 nM MTX and 7-OH-MTX using 1 ml of serum. A typical LC record is shown in figure 1. Overall extraction recoveries were 70% ± 5% (± SD) from 1-ml serum samples for both MTX and 7-OH-MTX. Peak heights (or areas) and both MTX and 7-OH-MTX concentrations were linearly related over the range of 0.020–100 μM. MTX concentrations up to 8 mM were determined by diluting the patient sera with drug-free serum to give concentrations within the range of the standard curve. The within-day assay precision for MTX gave coefficients of variation of 6.2% (n = 6; 22 nM) and 3.5% (n = 11; 1.85 μM). The between-day precision was 13.4% (n = 13; 100 nM) and 7.7% (n = 12; 1.85 μM). The precision (coefficient of variation) for 7-OH-MTX was estimated from three separate standard curves of five points each to range from 10% to 16%.

MTX analogs and potential metabolites (8) (table 1) did not interfere in the LC-UV assay of MTX and 7-OH-MTX. Sera from patients who were receiving 50 common drugs, excluding MTX, were also analyzed and the only drug with potential assay interference was hydralazine (retention time, 5.2 minutes) which was incompletely resolved from MTX (retention time, 6 minutes). Subsequent routine analysis of MTX patient sera showed a

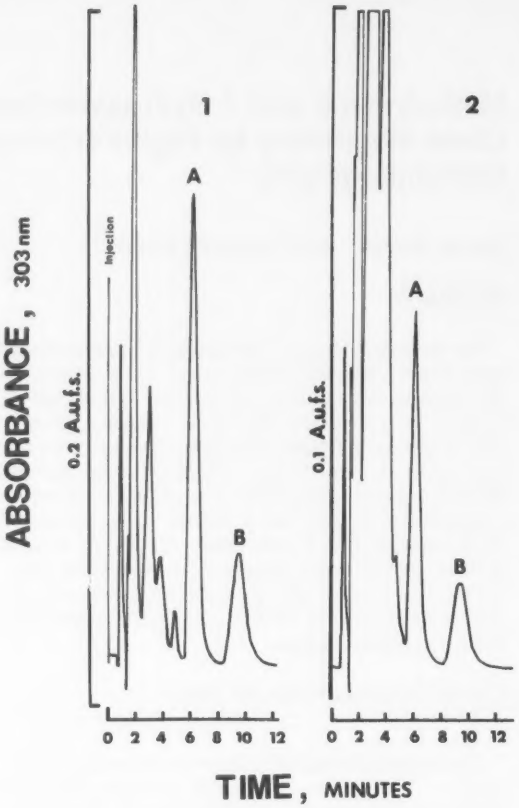

FIGURE 1.—Chromatogram of serum samples obtained from 1 patient (No. 1) at (1) 30 hrs and (2) 54 hrs after high-dose MTX infusion (9 g/m² over 6 hrs). Peak A = MTX; peak B = 7-OH-MTX. MTX levels at 30 and 54 hrs are 8.6 and 3.1 μM respectively, while 7-OH-MTX levels at the same times are 4.6 and 1.5 μM respectively.

hydralazine peak when this drug was administered concomitantly with MTX. This peak interfered significantly with MTX analysis in only two serum samples from the same patient; therefore, the hydralazine interference

TABLE 1.—Retention times obtained by direct injection and chromatography of drugs and MTX analogs under the conditions of the LC-UV assay

Compound	Retention time (mins)
Citrovorum factor	2.8
Hydralazine	5.2
MTX	6.0
7-OH-MTX	8.8
Trimethoprim	10.7
4-Amino-4-deoxy-N^{10}-methylpteroic acid	17.6

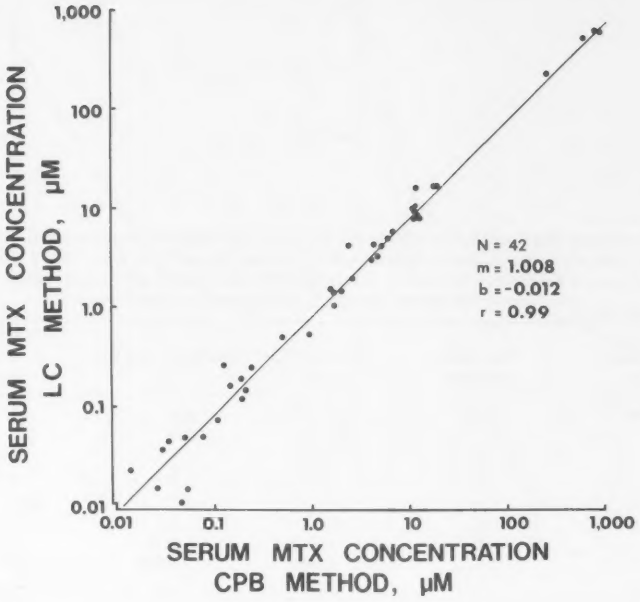

FIGURE 2.—Comparison of serum MTX concentrations obtained from analysis of 42 serum samples by both the competitive dihydrofolate reductase binding (CPB) and LC-UV assays, including the results contained in table 2. Note the logarithmic scale which tends to reduce the scatter upon visual inspection. The regression line is shown.

which was readily detected by the different retention time did not seriously affect the clinical utility of the LC-UV assay for MTX. The MTX metabolite, 4-amino-4-deoxy-N^{10}-methylpteroic acid (8), may also be analyzed by the LC-UV assay (analytic recovery of 60%), with an approximate limit of detectability of only 50 nM because of band-spreading at its rather long retention time. None of three randomly selected MTX sera showed measurable concentrations of this metabolite.

The LC-UV assay showed good correlation with an established specific dihydrofolate reductase binding assay (9) (fig 2). There was considerable scatter in the values < 100 nM, but high 7-OH-MTX levels did not appear to interfere in the competitive protein binding assay of MTX (table 2). 7-OH-MTX was present in all serum samples (table 2) with great variability relative to MTX. 7-OH-MTX/MTX ratios ranged from 0.01 to 40, increasing with time after dose. Serum concentration versus time curves for MTX and 7-OH-MTX in two selected patients (Nos. 1 and 2) are shown in figure 3; this figure demonstrates the large differences in individual MTX half-lives and 7-OH-MTX serum levels. The LC-UV assay was also applied to cerebrospinal fluid samples from four different patients (data not shown) and good agreement of MTX concentration data between this assay and the competitive protein binding assay was obtained. However, none of these samples contained detectable 7-OH-MTX levels (> 10 nM), indicating that the metabolite does not readily penetrate the blood-brain barrier.

In conclusion, this LC-UV assay which may be equally applicable to additional folate congeners with only minor modifications is suitable for therapeutic monitoring of serum levels of MTX and 7-OH-MTX above 10–20 nM.

TABLE 2.—Serum MTX and 7-OH-MTX levels in selected patients receiving moderate-dose MTX infusion (0.69 g/m² over 42 hrs) or high-dose MTX therapy (9 g/m² over 6 hrs) (MTX and 7-OH-MTX levels were determined by LC-UV assay, and MTX levels were additionally determined by the competitive protein binding [CPB] comparison assay [ref 9])

Patient No.	Hrs after infusion	MTX (CPB) (μM)	MTX (LC-UV) (μM)	7-OH-MTX (LC-UV) (μM)
1*	–3‡	850	606	7.9
	7	250	203	48
	30	11.6	8.6	4.6
	54	4.0	3.1	1.5
2*	0	790	637	1650
	11	11	7.9	32
	35	0.21	0.14	4.4
	57	0.030	0.036	2.0
3†	0	12.6	7.7	0.81
	24	0.20	0.12	0.10
4†	1	12.0	14.4	0.05
	6	4.1	4.3	1.0
	23	0.12	0.26	0.35
5†	0	11.0	9.3	2.4
	22	0.11	0.073	1.4
	0§	—	7.1	1.3
	7	—	1.5	2.5
	24	—	0.14	0.73
6†	24	2.0	1.5	9.9
	43	0.19	0.19	2.3
7†	14	6.8	5.8	28.7
	31	0.14	0.16	2.4
8†	0	—	8.0	6.6
	6	—	3.5	6.2
	17	—	0.78	3.6
	24	—	0.08	0.61

*High-dose MTX.
†Moderate-dose MTX.
‡Mid-infusion.
§2nd treatment.

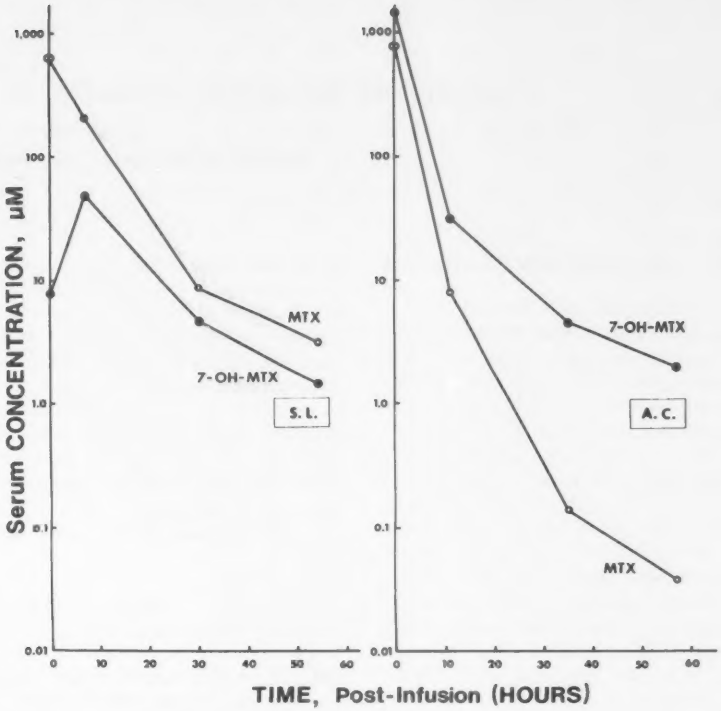

FIGURE 3.—Serum concentration vs time curves of MTX and 7-OH-MTX in patients 1 and 2 following high-dose MTX administrations (9 g/m^2 over 6 hrs).

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Tamoxifen in Metastatic Malignant Melanoma [1,2]

Frank L. Meyskens, Jr,* and Jane B. Voakes [3]

SUMMARY

Eight patients with advanced metastatic malignant melanoma were treated with tamoxifen at a dose of 10 mg orally twice a day for a minimum of 4 weeks. There was one complete response of skin disease occurring over a period of 40 weeks. This patient has remained disease-free for 8+ weeks. Two other patients demonstrated a partial but brief (4 weeks) shrinkage of gross subcutaneous disease. Three patients exhibited no response to the drug, but two patients had rapid acceleration of disease between the second and fourth weeks of treatment. Further exploration of the basis for these favorable and adverse responses is being pursued.

[Cancer Treat Rep 64:171–173, 1980]

The treatment of metastatic melanoma has been largely palliative. The remissions which are produced by combination chemotherapy are usually partial with short durations (1). New approaches to the treatment of melanoma are needed.

Clinical observations have long suggested that melanocytes are under endocrine control (2). Recent data indicate that melanoma tissue from some patients contains low levels of cytoplasmic estrogen, progesterone, androgen, and dexamethasone receptors (3,4). Short-term responses to diethylstilbestrol and to the weak antiestrogen, 6α-methylpregn-4-ene-3,11,20-trione, have yielded a small number of responses in patients with metastatic melanoma (5,6). We have, therefore, undertaken a trial of the nonsteroidal antiestrogen, tamoxifen, in the treatment of metastatic melanoma, and our results with the first eight patients treated with this compound are reported.

MATERIALS AND METHODS

Eight patients with histologically documented metastatic malignant melanoma were selected for treatment with tamoxifen. All but one of these patients had been heavily pretreated with multiple-drug chemotherapy. The patient who had not received prior cytotoxic therapy had refused chemotherapy from numerous institutions and was offered tamoxifen as an alternative agent. All patients were treated with tamoxifen given orally at a dose of 10 mg twice daily for a minimum of 4 weeks. No other hormonal, cytotoxic, immunologic, or radiation therapy was given while the patient was receiving tamoxifen. Physical examinations were performed every 2 weeks and chest roentgenogram, cbc, and liver function studies were obtained before treatment, monthly thereafter, and after treatment failure. Liver scans were obtained if the liver was clinically involved. Hormone receptor levels were not performed on the malignant tissue from these patients. The responses of individual patients are detailed in table 1.

RESULTS AND DISCUSSION

Three objective responses (37%) of metastatic malignant melanoma to tamoxifen were noted (table 1). A complete response has been obtained in one patient (No. 6) without prior chemotherapy with the total regression of 25 of 25 1–3-mm flat melanoma tumors without the appearance of disease elsewhere after 40 weeks of treatment. He has remained disease-free for 8+ weeks after achieving complete remission. A brief partial regression of subcutaneous tumors was observed in the other two patients (Nos. 1 and 7), between the second and sixth weeks of treatment. Both patients then progressed locally between the sixth and tenth weeks of treatment but without the appearance of other systemic disease. No response was noted in three patients after 4 weeks and the drug was discontinued. However, a rapid disease progression was noted in two patients between the second and fourth weeks of treatment. In one patient (No. 3), a slowly growing 8 × 10-cm area of the axilla was initially involved which rapidly advanced to involve the entire ipsilateral chest and flank. In another patient (No. 8), nasal mucosal disease and a 1 × 1-cm lung nodule

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TABLE 1.—Patient parameters and response

Patient No.	Sex	Age	Site and status of disease at start of tamoxifen therapy	Prior therapy	Response
1	M	50	Several large subcutaneous nodules (shoulder, 22 × 25 cm; chest, 22 × 15 cm; arm, 13 × 13 cm)	Bacillus Calmette-Guérin, DTIC, BCNU, hydroxyurea	Decrease of large forearm to 9 × 10 cm and chest lesions to 16 × 10 cm for 4 wks, with regrowth between 6th and 10th wks
2	M		Skin, lung	BCNU, hydroxyurea, DTIC, vincristine	No response
3	M	54	Skin and nodal disease slowly growing in axillary region (8 × 10 cm)	DTIC, actinomycin D	Rapid progression of skin lesions between 2nd and 4th wks to involve entire ipsilateral chest and flank
4	F	34	Lung, skin, liver	DTIC, methyl-CCNU, melphalan, hydroxyurea, Adriamycin	No response
5	F	44	Pelvic tumor	DTIC, actinomycin D	No response
6	M	62	Multiple (25) 1–3-mm facial skin metastases	None	Decrease to no lesions after 40 wks of treatment and in complete remission for 8+ wks
7	F	65	Several supraclavicular tumors (1 × 2, 1 × 1, 4 × 2, and 6 × 4 cm), lung nodule (1 × 1 cm)	DTIC, melphalan, Adriamycin, actinomycin D, hydroxyurea	Decrease of supraclavicular tumors to 1 × 0.5, 0, 3 × 1, and 4 × 3 cm for 4 wks
8	F	34	Nasal mucosa, lung	Actinomycin D, BCNU, hydroxyurea, DTIC	Appearance of massive hepatomegaly between 2nd and 4th wks; liver scan showing multiple large defects

remained stable, but the liver progressed from a normal span of 10-cm at the right midclavicular line to a total span of 38 cm. No further rapid progression or regression of these new lesions was noted in either patient when tamoxifen was stopped.

The mechanisms responsible for the favorable and possible adverse reactions may represent at least two effects of tamoxifen. The drug may be acting as an antiestrogen in the tumor and causing regression directly. However, in another study, two patients who did respond to estrogen therapy lacked estrogen receptors in their tumor tissue (5).

Alternatively, tamoxifen may work by altering follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels at the level of the hypothalamus or pituitary gland (7,8). Recently, Mather and Sato (9) have developed a serum-free medium to support murine melanoma growth, an essential component of which is a low concentration of FSH. We have also noted that colony formation obtained from fresh cells of human melanoma tissue can be supported in some instances by a similar FSH-containing medium, but that FSH at high levels is inhibitory.[4] Further studies of an established melanoma cell line show that the effects of FSH on cellular proliferation are concentration-dependent.[5]

If the effect of tamoxifen on melanoma represents a secondary effect of FSH or LH, a consistent change in these hormones may be seen. We are currently measuring changes in FSH and LH in patients with metastatic melanoma being treated with tamoxifen. These clinical results and further biologic studies of the role of FSH and LH in malignant melanoma may lead to new insights into this difficult neoplasm.

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Curative Radiotherapy for Localized Diffuse Histiocytic Lymphoma [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Ten newly diagnosed patients, with localized diffuse histiocytic lymphoma classified according to the Lukes-Collins classification, were evaluated for response to treatment with extended-field radiation. All classified tumors were of follicular center cell type (four large cleaved, four large noncleaved, and one small noncleaved). There were nine patients with stage I disease and one with stage II disease. The patient with stage II disease had only two sites of involvement. The disease was nodal in six patients and extranodal in four. All patients are alive without evidence of disease recurrence from 19 to 102 months since diagnosis (median survival, 58+ months). No differences were found in response or survival rates between histologic categories. No significant immediate or long-term complications have occurred. These results suggest that radiotherapy alone is curative for localized diffuse histiocytic lymphoma and may be superior to recently advocated chemotherapeutic approaches.

[Cancer Treat Rep 64:175–177, 1980]

Diffuse histiocytic lymphoma (DHL), one of the most aggressive malignant lymphomas, now appears to be curable in a percentage of patients. Bitran et al (1) reported a relapse-free survival rate of 80% with radiotherapy alone in pathologically staged patients with localized disease. DeVita et al (2) demonstrated that combination chemotherapy resulted in prolonged disease-free survival in almost 40% of patients with advanced disease. Recently, Miller and Jones (3) reported a relapse-free survival rate of 90% in ten patients with localized DHL treated with chemotherapy alone.[4] Their study has prompted us to report our long-term results in pathologically staged patients with localized DHL treated with extended-field radiotherapy alone.

MATERIALS AND METHODS

Between July 1970 and June 1978, ten patients who were diagnosed as having stage I or II DHL were seen in the Department of Therapeutic Radiology at our institute for primary treatment. All patients were carefully staged. The initial diagnostic evaluation included a complete medical history and physical examination, extensive laboratory and roentgenographic studies as previously described (4), bone marrow trephine biopsy, bipedal lymphography, and exploratory laparotomy. The extent of disease was classified according to the criteria of the Ann Arbor classification (5).

All patients were treated with extended-field radiation using techniques previously described (6). Briefly, radiation was initially delivered to all of the lymph nodes of the involved side of the diaphragm and subsequently to the contiguous chain on the other side of the diaphragm. The amount of radiation utilized was 5000 rads to the involved area; wherever possible, ie, areas in which tissue tolerance would not be exceeded (cervical nodes, axillae, etc), this area was boosted to 6000 rads. The uninvolved area, or prophylactic field, was treated to 4000 rads. Radiation was delivered at a dose of 150–175 rads daily with a 10-mev linear accelerator.

The pretreatment histologic material of all ten patients was reviewed and confirmed to be DHL according to the Rappaport classification (7). These cases were then classified according to the Lukes-Collins classification (8).

RESULTS

The ten patients ranged in age from 35 to 70 years (median age, 53). There were seven males and three fe-

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[4]Additional details and an updating of this study were kindly provided by Drs. T. P. Miller and S. E. Jones.

TABLE 1.—Clinical and pathologic characteristics of 10 patients with DHL

Patient— Age (yrs)	Sex	Lukes-Collins classification	Primary tumor site	Pathologic stage	Survival (mos)
35	F	Large noncleaved FCC	Axillary lymph node	I	102+
45	M	Large cleaved FCC	Submandibular lymph node	I	87+
62	M	Large noncleaved FCC	Inguinal lymph node	I	85+
41	F	Large cleaved FCC	Inguinal lymph node	I	49+
48	F	Large cleaved FCC	Stomach	I_E	39+
62	M	Large noncleaved FCC	Stomach	I_E	66+
56	M	Small noncleaved FCC	Stomach	I_E	46+
70	M	Large cleaved FCC	Stomach	I_E	19+
65	M	Unclassified	Waldeyer's ring (cervical lymph node)	II	85+
50	M	Large noncleaved FCC	Supraclavicular lymph node (high cervical lymph node)	I	31+

males. Nine patients were diagnosed as having stage I disease and one was diagnosed as having stage II disease. Sites of disease are indicated in table 1. The disease was nodal in six patients and extranodal in four.

The results of histologic typing of these cases of DHL according to the Lukes-Collins classification are shown in table 1. All of the classified cases were morphologically of Lukes-Collins B-cell type; four were large cleaved follicular center cell (FCC) lymphomas, four were large noncleaved FCC lymphomas, and one was a small noncleaved lymphoma. One tumor was unclassifiable due to the small amount of tissue available.

All patients are alive without evidence of disease recurrence 19–102 months from diagnosis. The median survival is 58+ months. Eight patients have been continuously disease-free for > 3 years (*see* table 1). No significant complications, immediate or long-term, have resulted from treatment.

DISCUSSION

Historically, localized histiocytic lymphoma has been treated with radiotherapy. The figures given for long-term control vary from 40% to 100% for stage I disease and from 25% to 65% for stage II disease (1,9,10). Much of this variability probably results because few studies include only pathologically staged patients. In the present study, where all patients have pathologically documented stage I or II disease, all patients currently enjoy prolonged disease-free survival. Similarly, in pathologically staged patients, Bitran et al (1) reported a 100% disease-free survival rate for stage I disease and a 65% disease-free survival rate for stage II disease. The minimum followup in our series is 19 months and the median followup is almost 5 years, suggesting that our patients are cured of their disease. The Chicago results (1) are also encouraging, although followup time is still short (minimum, 0.4 years; median, 2.4 years). All of our patients were treated with extended-field radiation while a number of radiation treatment plans were utilized by Bitran et al (1).

Recently, Miller and Jones (3) have treated ten patients with clinically localized DHL with intensive combination chemotherapy. All patients achieved complete remission. The median followup, as of June 1, 1979, is 36 months and to date only one patient has relapsed.[4] These excellent results have prompted them to advocate chemotherapy for localized DHL.

Our series and that of Miller and Jones (3) are too small to allow conclusions on the best approach for DHL of limited extent. There are different types of limited DHL. By chance, all of the patients in our series had two or less sites of involvement. The patients in the study by Miller and Jones (3) generally had more than two sites of involvement. It is possible that radiotherapy is indicated for limited stage I and II disease but that chemotherapy is required for more extensive stage II disease. Bitran et al (1) noted relapses following radiotherapy only in patients with stage II disease with more than three sites of involvement. Furthermore, in advanced DHL treated with chemotherapy alone, relapse is common in sites of initial bulky disease. Radiotherapy may be particularly indicated in such circumstances.

In addition, the short-term results of treatment will have to be weighed against the late complications. Second neoplasms have been reported more frequently in patients treated with chemotherapy alone or combined with radiotherapy than in patients treated with radiotherapy alone (11,12). A further consideration is that in advanced DHL, combination chemotherapy has been reported to

be most effective in patients with the large cleaved FCC histologic subtype and considerably less effective in patients with other subtypes (13). It is unknown whether this will also be the case in localized DHL. In our small series, both large cleaved and large noncleaved FCC types appear to be equally responsive to radiotherapy.

Our results with the extended-field technique suggest that radiotherapy alone is usually curative in DHL of limited extent. Assuming that the promising early results of Miller and Jones (3) are substantiated by continued followup, a randomized clinical trial comparing intensive combination chemotherapy with extended-field radiotherapy will be required to determine the best approach for the individual patient with localized DHL.

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Phase II Trial of Vindesine in Patients With Malignant Melanoma[1,2]

Fernando J. Camacho, Charles W. Young, and Robert E. Wittes[3,*]

Malignant melanoma is relatively resistant to chemotherapy, and the need for new, more active agents is quite evident (1). Vindesine is a new, semisynthetic vinca alkaloid with a broad range of activity in murine hematologic and solid tumors. The established vinca alkaloids, vincristine and vinblastine, have some activity in malignant melanoma, with response rates in the 10%–15% range (1). Other centers have reported some activity of vindesine in this disease (2,3). In phase I trials conducted at our institution (4), a dose of 3–4 mg/m^2 every 7–14 days was considered appropriate for phase II study. Accordingly, we undertook to define the level of activity of this agent in malignant melanoma.

PATIENTS AND METHODS

Forty-seven patients with histologically proven malignant melanoma were entered in this study. The extent of disease was evaluated in each patient prior to entry in the study by physical examination, chest roentgenogram, cbc and platelet count, and 12-channel screening profile. Scintiscans and additional radiographic studies were obtained when indicated.

Vindesine was given at an initial weekly dose of 2–4 mg/m^2 by iv injection. The dose was increased or decreased weekly as toxicity permitted. Patients were considered adequate if they received at least two doses or developed dose-limiting toxic effects. A partial response was defined as a $>$ 50% reduction in the sum of the product of the largest perpendicular diameters of measurable lesions without progression in any lesion or the appearance of new lesions for at least 1 month. A minor response was defined as an objective response not meeting the criteria of a partial response.

RESULTS

Seven patients received inadequate trials of therapy: two were lost to followup after the second dose and five received only one treatment because of their very poor general physical condition. The characteristics of the 40 evaluable patients are shown in table 1. Data concerning response to therapy and toxicity are shown in table 2. All of the patients had an excellent Karnofsky performance status with a mean of 80. Only five patients had a performance status of $<$ 70. Although 34 of 40 patients (85%) had received some prior nonsurgical therapy, only 25 of 40 (62%) had received prior chemotherapy. The drug was well-tolerated except for the expected neurotoxicity (paresthesias, jaw pain, weakness, and constipation); the myelosuppression was rather mild. In patients who received more than a few doses of therapy, the frequency of drug administration was occasionally decreased to every other week in an effort to minimize the neurotoxicity. Of note is the apparent increased marrow toxicity of vindesine occurring in patients who had received prior chemotherapy (64%) compared to those without previous drug exposure (27%). The occurrence of alopecia was not meticulously recorded and is therefore probably underestimated in the present study.

The therapeutic results were disappointing. Only four patients experienced partial responses with vindesine therapy. Interestingly, and probably coincidentally, all four patients had received prior chemotherapy. Two of these patients had stage IIIA disease, one had stage IIIB disease, and one had stage IV disease. The durations of response were 2 and 11+ months for the patients with stage IIIA disease, 1 month for the patient with stage IIIB disease, and 4 months for the patient with stage IV disease.

DISCUSSION

Vindesine produced four responses among 40 adequately treated patients with metastatic malignant melanoma (response rate, 10%; 95% confidence limits, 3.7%–24%). Except for one patient, all responses were of very short duration. Although the majority of our patients had received prior chemotherapy, none of the 15 previously untreated patients responded. The activity seen in this

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TABLE 1.—Characteristics of 40 adequately treated patients

Characteristic	Prior chemotherapy	No prior chemotherapy	Totals
Total No. of patients	25	15	40
Males	17	8	25
Females	8	7	15
Age in yrs			
Mean	53	47	51
Median	54	42	50
Performance status			
Mean	80	90	80
Range	50–100	70–100	50–100
No. of patients with—			
Disease stage			
IIIA (nonregional skin, subcutaneous)	4	2	6
IIIB (single visceral site)	7	8	15
IV (multivisceral)	14	5	19
Primary tumor site			
Head and neck	5	0	5
Trunk	10	9	19
Extremities	8	3	11
Unknown	2	3	5
Prior therapy			
Radiation therapy	9	3	12
Immunotherapy	14	6	20
Extent of disease			
Lungs	15	10	25
Liver	3	2	5
Brain	4	2	6
Skin/subcutaneous	21	10	31
Nodes	2	4	6
Bone	3	1	4
Other	4	1	5
No. of vindesine doses			
2	3	0	3
3–4	6	7	13
5–6	5	3	8
7–8	3	2	5
> 8	8	3	11
Total vindesine dose (mg)			
8–20	15	9	24
21–40	7	6	13
> 40	3	0	3

TABLE 2.—Response to therapy and toxic effects

	No. of patients		
	Prior chemotherapy	No prior chemotherapy	Totals
Total	25	15	40
Response			
Partial	4	0	4
Minor	1	2	3
Stable	2	5	7
Progression	18	8	26
Toxic effects			
Mucositis	3	2	5
Nausea and vomiting	6	8	14
Constipation	9	4	13
Diarrhea	2	1	3
Paresthesias	13	9	22
Weakness and malaise	14	8	22
Jaw pain	2	1	3
Abdominal pain	4	6	10
Hoarseness	1	2	3
Fever and chills	6	2	8
Alopecia	5	4	9
Anemia (hemoglobin < 12 g/100 ml)	16	4	20
Wbc count nadir (cells × 10^3/mm^3)			
3.0–3.9	5	3	8
2.0–2.9	5	2	7
< 2.0	5	0	5
Thrombocytopenia (< 150,000 cells/mm^3)	2	0	2

study confirms the response rate of 16% reported by Smith et al (3) in a smaller group of patients. Vindesine, therefore, does not seem to offer any obvious advantage over the other vinca alkaloids, methyl-CCNU, or DTIC. Further trials of this agent in the present dose and schedule in patients with malignant melanoma appear unwarranted.

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Phase II Trial of 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA) in Patients With Metastatic Hypernephroma[1,2]

Robert J. Schneider, Thomas M. Woodcock, and Alan Yagoda[3,4,*]

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA), a new acridine derivative thought to function by intercalation and external binding with DNA, was found in preclinical studies to possess antitumor activity against various murine tumors (1). In phase I clinical trials responses were observed in a variety of neoplasms, particularly in leukemia and breast cancer (2–5). Since renal cell carcinoma is a chemotherapeutically resistant tumor, a disease-oriented phase II trial of AMSA in patients with objectively measurable metastatic hypernephroma was initiated and forms the basis of this report.

MATERIALS AND METHODS

Twenty-one patients with metastatic hypernephroma received AMSA. Pathologic material was reviewed in 20 patients by the Department of Pathology at our institute. The diagnosis of hypernephroma was accepted in one patient on the basis of typical findings by nephrotomograms, selective renal arteriograms, and computerized transaxial tomograms. Each patient had a complete medical history and physical examination, and all measurable lesions were evaluated independently by two investigators. Automated blood cell and platelet counts were obtained weekly; weight, performance status (Karnofsky scale), screening biochemical profiles (including liver function tests), and chest roentgenograms were obtained every 3 weeks; and appropriate diagnostic tests needed to evaluate responses (ie, radionuclide scans and computerized transaxial tomograms) were obtained every 3–6 weeks.

Criteria for patient selection included acceptable hematologic status (an initial wbc and platelet count of $\geq$ 4500 and 175,000/mm^3 respectively), the presence of bidimensionally measurable lesions, a performance status of $\geq$ 50%, and no evidence of significant hyperbilirubinemia or renal dysfunction.

AMSA was administered as an iv infusion over a 30-minute period every 3 weeks. The initial dose was 120 mg/m^2 for patients with good bone marrow reserve, while the initial dose was 90 mg/m^2 for patients who received extensive irradiation or had multiple courses of combination chemotherapy. Dose escalation by 30 mg/m^2 occurred with a wbc count nadir of $\geq$ 3000/mm^3 and a platelet count nadir of $>$ 100,000/mm^3. Dose reduction from 120 to 150 mg/m^2 by 30 mg/m^2 occurred with a wbc count nadir of $<$ 3000/mm^3 and a platelet count nadir of $<$ 100,000/mm^3.

An adequate trial was defined as two doses with myelosuppression or 120-mg/m^2 doses with rapid disease progression and 1-month survival. Response criteria included *partial remission*—a $\geq$ 50% reduction in the sum of the products of the diameters of all measurable lesions for $>$ 1 month; *minor remission*—a 25%–49% decrease in measurable lesions for $>$ 1 month or a $>$ 50% decrease for $<$ 1 month; and *disease progression*—mixed response, stable disease ($<$ 25% increase or decrease in tumor size), and a $\geq$ 25% increase in the sum of the products of the diameters of all measurable lesions.

Patient Characteristics

There were 18 males and three females with a median age of 62 years (range, 22–75) and a median performance status of 70% (range, 50%–90%). The median time from symptoms to diagnosis, from diagnosis to metastases, and from diagnosis to protocol was 1 month (range, 0–12), 0 months (range, 0–69), and 16 months (range, 0–31) respectively. Seventeen patients had had no prior chemotherapy. Six patients had had prior irradiation, one had had immunotherapy, and six had had hormonal therapy. All prior treatment had been discontinued for at least 30 days before the initiation of this protocol.

Primary indicator lesions included pulmonary metastases (14 patients), nodes (one), and abdominal tumors, measured by computerized transaxial tomography (six).

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[2]Supported by contract N01-CM-57043 (Division of Cancer Treatment) and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Developmental Chemotherapy Service (*R. J. Schneider* and *T. M. Woodcock*) and Solid Tumor Service (*A. Yagoda*), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[4]We wish to gratefully acknowledge the technical and secretarial assistance of Jane Howe and Isa Irvin, respectively.

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TABLE 1.—Hematologic toxicity of AMSA

Dose (mg/m²)	No. of patients entered	No. of courses	Total % of patients with toxic effects	Leukopenia*			Thrombocytopenia†		
				% of patients	Nadir (cells × 10^3/mm³) Median (range)	Day	% of patients	Nadir (cells × 10^3/mm³) Median (range)	Day
90	6	11	83	83	2.4 (1.4–3.1)	9	33	88 (54–122)	11
120	17	25	76	76	2.7 (1.1–4.0)	13	12	113 (55–149)	7
150	10	10	90	90	2.6 (1.3–3.8)	8	10	84 (84)	6
180	1	2	100	100	0.3	10	100	66	12

*≤ 4000 cells/mm³.
†< 150,000 cells/mm³.

Measurable lesions among the 21 patients included pulmonary (14), nodal (three), hepatic (three), intra-abdominal (seven), subcutaneous (four), and abnormal liver function tests (six).

RESULTS

All patients had an adequate trial. The only mixed response occurred in a patient with malignant hepatomegaly and pulmonary metastases who had > 75% reduction in the size of pulmonary nodules with active disease progression in the liver observed by physical examination. However, response was noted in the pulmonary lesions 2 days after a pneumothorax occurred from a percutaneous pulmonary needle aspiration. Regression of pulmonary disease persisted for 3 months, at which time regrowth was noted. There were no other cases of objectively measurable responses to AMSA. The median survival time from initiation of protocol to followup or death, as of July 1979, was 6 months (range, 1–9+).

The median number of doses was two: two patients received one dose, 13 received two doses, two received three doses, two received four doses, and one each received five and six doses. Hematologic toxicity was dose-limiting (*see* table 1). Myelosuppression was observed in patients at doses of 90 mg/m^2 (83%), 120 mg/m^2 (76%), and 150 mg/m^2 (90%). Leukopenia was more frequent than thrombocytopenia; there was no evidence of cumulative hemopoietic toxicity. There was no evidence of mucositis or gastrointestinal disturbances, and there were only rare instances of anorexia or nausea.

DISCUSSION

AMSA has < 15% antitumor activity in advanced, metastatic hypernephroma and seems to have no role in the treatment of this tumor.

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Combination Chemotherapy for Advanced Squamous Cell Carcinoma of the Head and Neck[1]

Brief Reports and Preliminary Communications

Jahangir Raafat and Martin W. Oster[2,3,*]

SUMMARY

Fifteen patients with advanced squamous cell carcinoma of the head and neck were treated with the six-drug Price-Hill regimen (vincristine, bleomycin, methotrexate, hydrocortisone, 5-fluorouracil, and leucovorin). Only two partial remissions were obtained, and the regimen produced moderate toxicity. Our results are not as favorable as those of Price and Hill.

[Cancer Treat Rep 64:187–189, 1980]

Squamous cell carcinoma of the head and neck has long been known to show some responsiveness to various chemotherapeutic agents (1). Recently, several reports have suggested that, as with lymphomas, small cell cancer of the lung, and breast cancer, combination chemotherapy produced improved response rates (1,2). Among the most encouraging reports have been studies from the Royal Marsden Hospital (London, England) using a six-drug combination known as the Price-Hill regimen (*see* fig 1) (3,4). Excellent response rates with minimal toxicity have been reported by Price, Hill, and their colleagues. For that reason, we conducted a pilot study using the Price-Hill regimen to confirm their results. Unfortunately, our findings are most disappointing.

MATERIALS AND METHODS

Fifteen patients with histologically proven advanced squamous cell carcinoma of the head and neck were entered in the study. As shown in table 1, 11 patients were male, and the patients' ages ranged from 28 to 82 years (median, 60). Only two patients had received no prior therapy, one of whom (No. 5) had received radiotherapy when she was 1 year old for bilateral retinoblastoma. Of the 13 previously treated patients, all had had prior radiotherapy and eight had had prior surgery. No patient had received prior chemotherapy of any kind. Table 1 lists the sites of disease in these patients. All patients were at least partially ambulatory and had a fair to adequate nutritional state at the time of entry in this study.

Chemotherapy consisted of the Price-Hill regimen as outlined in figure 1. The drugs were given as follows: vincristine at a dose of 2 mg iv at 0 hour; bleomycin at a dose of 60 mg as a 6-hour infusion from 12 to 18 hours; methotrexate at a dose of 100 mg iv at 12, 15, and 18 hours; hydrocortisone at a dose of 500 mg iv at 12 and 18 hours; 5-fluorouracil at a dose of 500 mg iv at 18 hours; and leucovorin (folinic acid) at a dose of 15 mg orally or im at 26, 32, 38, and 44 hours.

Treatment cycles were repeated every 3–4 weeks. Four patients received one cycle, seven received two cycles, and four received three or more cycles. All patients were considered evaluable.

Responses were defined as follows: *complete response*—the disappearance of all lesions for > 4 weeks; *partial response (PR)*—a > 50% tumor regression (product of two diameters) for > 4 weeks; *stable disease*—no change in any indicator lesion; and *disease progression*—an increase in indicator lesions or the appearance of new tumors.

RESULTS

Two patients (13%) had a PR with durations of 5 and 28 weeks. Two patients (13%) had stable disease for periods of several weeks only. Eleven patients (73%) had disease progression. All patients died within several months after disease progression or relapse after PR or stable disease.

The toxic effects of this regimen were moderate. Three patients had severe leukopenia (wbc counts < 400/mm^3) with associated infections: one patient died from sepsis. Three patients developed severe stomatitis, while most of the other patients experienced mild or no stomatitis. There was no severe nausea and vomiting, cardiopulmonary toxicity, or nephrotoxicity.

[1]Received Aug 9, 1979; revised Oct 1, 1979; accepted Oct 11, 1979.

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TABLE 1.—Patient characteristics and results

Patient No.	Age/sex	Primary tumor	Previous therapy	Site of disease recurrence	No. of treatments	Response	Duration of response (wks)
1	82/M	Primary unknown cervical nodes	Radiotherapy	Cervical nodes, chest wall	1	Disease progression	—
2	79/M	Buccal mucosa	Radiotherapy, cryosurgery	Local	4	Partial	5
3	47/M	Hard palate	Surgery, radiotherapy	Widespread metastases (nodes, lung, bone)	2	Disease progression	—
4	79/M	Left tonsil	Radiotherapy, surgery	Local	1	Disease progression	—
5	28/F	Bilateral, frontal, sinus	Radiotherapy 27 yrs ago for bilateral retinoblastoma	—	2	Disease progression	—
6	70/M	Larynx	Surgery, radiotherapy	Chest wall, cervical nodes	1	Disease progression	—
7	45/M	Nasopharynx	Radiotherapy	Orbit, cervical nodes	2	Disease progression	—
8	59/M	Base of tongue	—	—	2	Stable disease	—
9	63/F	Left tonsil	Radiotherapy	Local and base of tongue	1	Disease progression	—
10	60/M	Larynx	Surgery, radiotherapy	Local and cervical nodes	3	Stable disease	3
11	74/F	Anterior tongue	Surgery, radiotherapy	Local	2	Disease progression	—
12	57/M	Base of tongue	Radiotherapy, surgery	Local, cervical nodes	2	Disease progression	—
13	62/F	Right tonsil	Radiotherapy, surgery	Local, cervical nodes	2	Disease progression	—
14	39/M	Nasopharynx	Radiotherapy	Local, cervical nodes	3	Disease progression	—
15	63/M	Unknown primary, cervical nodes	Radiotherapy	Cervical nodes	6	Partial	28

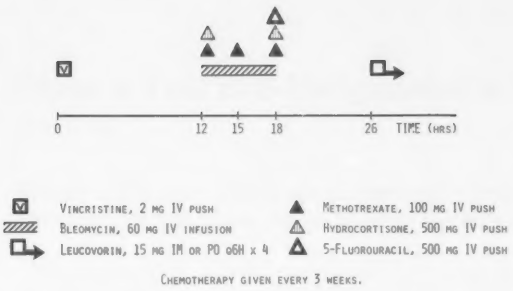

FIGURE 1.—Chemotherapy schedule for patients with advanced squamous cell carcinoma of the head and neck.

DISCUSSION

Our results with the Price-Hill regimen were disappointing with only a 13% PR rate which was only briefly maintained. Moderate toxicity also occurred with this regimen. This contrasts with the results reported most recently by Price et al (4). Forty-three of the 68 patients (63%) treated with the Price-Hill regimen responded. Patients with prior radiotherapy had a lower (56%) response rate than those who had received no previous radiotherapy (72%). Toxicity from this regimen was felt to be minimal.

Although most of our patients had received prior radiotherapy and the number of patients in our series is small, we feel that our disappointing results do not confirm those of Price and Hill. Dalley (5), working at the same institution as Price and Hill, has likewise implied disappointing results obtained with the Price-Hill regimen, although she does not give details. Clearly, others should report their experiences with this regimen. We have abandoned further studies with this regimen and are currently evaluating *cis*-dichlorodiammineplatinum(II)-containing regimens, which have produced encouraging results in several early trials (2,6).

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Phase II Trial of 6-Thioguanine in Metastatic Breast Cancer[1]

Brief Reports and Preliminary Communications

Kishan J. Pandya,[2] Douglass C. Tormey,[2,*] Thomas E. Davis,[2] Geoffrey Falkson,[3] Tarit K. Banerjee,[4] and John Crowley[2,5]

6-Thioguanine is a purine antagonist used predominantly in the treatment of acute myelocytic leukemia. It has not been adequately evaluated in patients with breast cancer (1). Muss et al (2) recently reported that of 31 patients with advanced breast cancer treated with a combination of vinblastine and 6-thioguanine two had a partial response and 12 others had stable disease. The patients were treated with vinblastine at a dose of 5 mg/m^2 by iv push on Day 1, and then with 6-thioguanine at a dose of 100 mg/m^2 orally, divided into two daily doses on Days 2–6.

The present study was undertaken to evaluate the effectiveness of 6-thioguanine as a single agent in previously treated patients with advanced breast cancer. Since the drug is an antimetabolite with a short plasma half-life, we decided to administer it on a single daily dose schedule rather than on a single-dose intermittent schedule. Additionally, the long cell cycle time of breast cancer cells (3) suggests that therapeutic levels should be maintained for at least 10–14 days to maximize cell-kill.

MATERIALS AND METHODS

Twenty-three patients with advanced breast cancer were treated with 6-thioguanine between June 1976 and May 1978. All patients had a histologically documented diagnosis of mammary carcinoma with recurrent metastatic disease. All patients had had prior exposure to at least one chemotherapy program, including exposure to Adriamycin and an alkylating agent. Most of the patients were exposed to multiple hormonal and chemotherapeutic agents (mean, 6.6; range, two to 14). Twelve of the patients had responded to prior chemotherapy regimens. Some patients had also received prior palliative radiotherapy. All patients in the study had an Eastern Cooperative Oncology Group performance status of 0, 1, or 2, evidence of progressive disease with measurable parameters, and a wbc count of $> 4000/mm^3$ and a platelet count of $> 100,000/mm^3$.

6-Thioguanine was given at a dose of 2.5 mg/kg orally as a single daily dose, rounded to the nearest 20 mg. Weekly blood cell counts were obtained. The dose was reduced to 50% if the wbc or platelet counts were < 4000 and $100,000/mm^3$ respectively, and the drug was omitted completely if the blood cell counts were < 2500 and $75,000/mm^3$ respectively.

Clinical and/or roentgenographic measurements were used to define response. The response criteria used were those defined by the International Union Against Cancer (4). For nonresponding patients, stable disease was defined as at least 56 days of receiving treatment without evidence of disease progression. The sites of measurable disease were evaluated at monthly intervals. Patient characteristics, results of prior therapy, and responses to therapy with 6-thioguanine are shown in table 1.

RESULTS

Three of the 23 patients died within 18 days after the start of treatment: one died from progressive disease and two died from drug-related toxicity. A partial response was observed in one patient, with a response rate of 4.3% (95% confidence limit, 0.3%–21.9%). This patient had a disease-free interval of 48 months and a history of responses to all prior chemotherapy and hormonotherapy received. Stable disease was seen in an additional three patients, lasting 64, 74, and 95 days. The duration of response in the one patient with a partial response was 221 days.

The four patients who had a partial response or stable disease had a median survival time of 174 days, while those who had continued disease progression had a median survival time of only 66 days. For all 23 patients, the median time to disease progression was 27 days and the median survival time was 87 days.

All of the patients received full optimal doses except

[1]Received Aug 9, 1979; revised Oct 15, 1979; accepted Oct 23, 1979.

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[4]Marshfield Clinic, WI.

[5]We greatly appreciate the invaluable assistance of Jane Graf in data management.

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TABLE 1.—Patient characteristics, results of prior therapy, and responses to 6-thioguanine

Parameters	No. of patients (%)
Performance status*	
0 (normal activity)	10(43)
1 (symptoms but ambulatory)	5(22)
2 (bedridden < 50% of time)	8(35)
Age in yrs (range, 26–69)	
≤ 50	8(35)
> 50	15(65)
Dominant disease sites	
Visceral	13(56)
Osseous	5(22)
Soft tissue	5(22)
Disease-free interval in mos (mean, 20.5)	
0 (stage IV at diagnosis)	5(22)
2–36	12(52)
> 36	6(26)
Results of prior drug therapy (mean No. of agents, 6.6; range, 2–14)†	
Response	12(52)
Stable disease	4(17)
Response to 6-thioguanine	
Partial response	1(4.3)‡
Stable disease, no change	3(17.4)§

*According to Eastern Cooperative Oncology Group.
†Includes hormonal and chemotherapeutic agents.
‡95% confidence interval, 0.3%–21.9%.
§Partial response + no change; 95% confidence interval, 6.2%–38.7%.

one, who received 80% of the full optimal dose. Toxic effects are shown in table 2. The hematologic toxicity was moderate to severe, and two patients died of drug-related toxic effects. Both of these patients had severe neutropenia and thrombocytopenia; one had definite gastrointestinal bleeding and the other had probable bleeding and sepsis. The majority of the patients had no nausea or vomiting. Four patients had moderate or severe gastrointestinal toxic effects. One patient was noted to have multiple 2–3-mm erythematous lesions over the right arm, scapula, and chest, which were suggestive of a drug reaction.

TABLE 2.—Toxicity

Parameters	No. of patients with toxicity grade*—					
	0	1	2	3	4	5
Wbc count	4	3	8	6	1	1
Platelet count	11	1	4	1	4	2
Nausea and emesis	14	5	2	2	0	0

*Eastern Cooperative Oncology Group grading: *wbc count (cells/mm³)*—0 = ≥ 4500, 1 = 3000–< 4500, 2 = 2000–< 3000, 3 = 1000–< 2000, 4 = < 1000, 5 = < 1000 at time of death; *platelet count (cells/mm³)*—0 = ≥ 130,000, 1 = 90,000–< 130,000, 2 = 50,000–< 90,000, 3 = 25,000–< 50,000, 4 = < 25,000, 5 = < 25,000 at time of death; *nausea and emesis*—1 = nausea, 2 = controllable nausea and emesis, 3 = intractable nausea and emesis.

DISCUSSION

The daily single-dose administration of 6-thioguanine in this study resulted in a very low objective response rate. A very few additional patients had stable disease. All patients in this study had advanced disease which had become refractory to prior chemotherapy and, in most cases, hormonotherapy. In this group of patients, the median time to disease progression was < 4 weeks and the median survival time was < 3 months. For these reasons, our patients represented a very poor-risk group and a high response rate would not have been expected. However, all of the patients entered in this study had a good performance status. It is possible that this drug might produce better results in untreated patients; however, we conclude that it is unlikely that much benefit would accrue from the incorporation of 6-thioguanine into earlier treatment programs employing combination chemotherapy.

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LETTERS

Changes in Hair Pigmentation Associated With Cancer Chemotherapy

Cancer therapy is increasingly effective in improving the survival and the quality of life in many patients. Contemporary cancer specialists use a greater variety of chemotherapeutic agents more frequently than their predecessors. For these reasons the side effects of such therapy have become increasingly variable and to some extent more unacceptable, both to the physician and the patient. A woman who came to our department for treatment of ovarian cancer brought to our attention what appears to be an unusual complication of cancer chemotherapy: a change in hair color. We present the clinical details of this and another case in which a definite change in hair pigmentation was noted following regrowth after cytotoxic drug-induced alopecia.

Case 1

A Caucasian man aged 68 years was treated with combination chemotherapy for a malignant melanoma of the soft palate. Following the surgical removal of the tumor he received courses of CCNU, bleomycin, and cyclophosphamide every 5–6 weeks for a period of 1 year. Alopecia developed after the third and fourth cycles of chemotherapy. His hair regrew when treatment was changed to DTIC alone. His hair had been a dull blond color prior to treatment and this had not changed since adolescence. However, when the hair regrew during DTIC chemotherapy it became progressively darker and stabilized at a dark brown color. He is not particularly perturbed by this change and remains well and in remission at 5 years.

Case 2

A Caucasian woman aged 40 years was treated with cyclic combination chemotherapy for a carcinoma of the ovary. She received vincristine, Adriamycin, 5-fluorouracil, and cyclophosphamide at monthly intervals for 1 year. Complete loss of body and scalp hair followed the first course of treatment. Her hair regrew after the cyclic treatment was spaced to 3-month intervals. Post-alopecia scalp and body hair was dark brown compared to its previous light mousey color. The patient was upset by the change in her appearance and was frequently aggravated by the reactions of her family and friends. She remains well and in complete remission 3 years later, her hair is still dark brown, and she is still unhappy about this change.

Alteration in skin pigmentation has been described in association with acute X-irradiation dermatitis (1) but to our knowledge post-chemotherapy-induced change in hair pigmentation has not been previously reported in the literature. In our department a wide cross-section of adult and pediatric tumors are treated with chemotherapy. We speculate from preliminary enquiries that between 10% and 20% of patients notice a change in hair pigmentation following regrowth after cytotoxic drug-induced alopecia. A detailed study has been established to verify this.

The emotional implications for the patient of such therapy-associated changes in their appearance cannot be ignored. This is likely to be variable, as exemplified by the two cases described. Local sociocultural factors will certainly intervene. Recent reports suggest that drug-induced alopecia may be avoided by the use of a scalp tourniquet (2–4). It would be interesting to know if such preventive therapy also eliminates chemotherapy-related hair pigmentation changes. We feel that patients should be forewarned of these drug-associated effects, uncommon though they may be.

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Response to Maytansine in a Patient With Malignant Thymoma

This case report details the response of a patient with malignant thymoma to maytansine. A 36-year-old black

.nan was diagnosed in September 1977 as having an inoperable malignant thymoma of mixed epithelial and lymphoid variety. The patient had no signs or symptoms of myasthenia gravis throughout the course of his disease. Following cobalt radiotherapy (March 7, 1978, through April 18, 1978) to the anterior and posterior chest via 11 × 14-cm fields to a dose of 4600 rads (to the mid-chest), the patient remained asymptomatic through June 1979. He then developed a cough and shortness of breath on minimal exertion. His work-up at that time revealed equivocal changes in the mediastinum possibly related to recurrent disease or prior radiotherapy. Abnormal liver function studies prompted the performance of a liver scan which revealed a filling defect in the right lobe. A percutaneous liver biopsy revealed a tumor identical to the original lesion diagnosed as malignant thymoma. In June 1979 the patient began receiving combination chemotherapy with Adriamycin (50 mg/m^2) and *cis*-dichlorodiammineplatinum(II) (50 mg/m^2) iv every 3 weeks. He continued treatment through August 1979, at which time it became apparent that his disease was progressing in spite of therapy. His liver then measured 22 cm below the right costal margin at the mid-clavicular line. In October 1979 the patient began receiving chemotherapy with maytansine at a dose of 0.75 mg/m^2. After 4 weeks of therapy (a total of 6.0 mg of maytansine), a dramatic diminution in the size of the central metastatic nodule within the liver became apparent. A repeat liver scan was performed on November 7, 1979, which revealed a 50% decrease in the volume of the central metastasis within the right lobe of the liver. The liver now measures 10 cm below the right costal margin.

This represents the second patient with malignant thymoma (1) to have a significant response to chemotherapy with maytansine.

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November 30, 1979

ANNOUNCEMENTS

Research Conference on Head and Neck Oncology

Rosslyn, Virginia, September 8–10, 1980

The Division of Cancer Treatment of the National Cancer Institute, National Institutes of Health (Bethesda, MD), is sponsoring a multidisciplinary conference on head and neck oncology to be held in Rosslyn, Virginia, September 8–10, 1980. The purpose of the conference is to provide a forum for the discussion of recent experimental and clinical research in head and neck cancer and to assess areas of research which should have high priority for future development.

Participation will be limited to persons actively engaged in either experimental or clinical research. Conference topics will include recent clinical trials, new diagnostic and therapeutic approaches employing surgery, radiation therapy, chemotherapy, immune modulation, or other investigational modalities, and laboratory investigations dealing with immunology, virology, cell culture, and animal tumor-model development. Conference participants may elect to present recent research or research in progress or to participate in discussions. *Interested investigators should contact:* Gregory T. Wolf, MD, Special Assistant for Surgical Oncology, Landow Bldg, Rm 8C17, National Cancer Institute, Bethesda, MD 20205 (telephone: 301-496-4844).

Chemotherapy Foundation Symposium IV

New York, New York, October 22–24, 1980

Chemotherapy Foundation Symposium IV entitled "Optimum Strategies for Clinical Cancer Chemotherapy" is scheduled for October 22–24, 1980, at the Barbizon Plaza Hotel in New York, NY. A national and international faculty of clinical investigators and clinicians will participate.

The 3-day program, under the chairmanship of Dr. Ezra M. Greenspan, is presented by The Division of Medical Oncology, The Department of Neoplastic Diseases, and The Page and William Black Post-Graduate School of the Mt. Sinai School of Medicine of the City University of New York. Symposium registrants will be eligible for AMA Category I credits. The fee is $200 for physicians and $75 for house staff and fellows.

***For further information and registration forms, write to:* Director, The Page and William Black Post-Graduate School of Medicine, Mt. Sinai School of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029 (telephone: 212-650-6772).**

2nd Rome International Symposium on "Biological Bases and Clinical Implications of Tumor Radioresistance"

Rome, Italy, September 21–24, 1980

This symposium, organized by an international scientific committee, will be held in Rome, Italy, on September 21–24, 1980, under the sponsorship of the Committee for Radiation Oncology Studies/National Cancer Institute, the European Organization for Research on Treatment of Cancer, and the Società Italiana di Cancerologia. The aim of the symposium is to review the current status of radiotherapy, to develop a critical evaluation or the "growing points" in the field, and to see what biologists and radiotherapists think should happen in the treatment of cancer. The topics will include: Biological Factors Influencing Radioresistance; Approaches to Exploiting Biological Phenomena; and Current Status and Growing Points of Radiotherapy (including unconventional dose delivery, hypoxic cell sensitizers, hyperthermia, combined drugs and radiation, and high LET radiation).

The program includes invited keynote addresses and lectures, a poster session, and a commercial exhibition. *For further information write to:* 2nd Rome Symposium, Istituto Medico e di Ricerca Scientifica, Via Luigi Bodio 58, 00191 Rome, Italy.

Oncology/Hematology Fellowships

Starting July 1980, 2–3 year programs in either Oncology or Hematology or both fields will be available at the University of Wisconsin—Milwaukee campus. Excellent in- and out-patient clinical experience as well as research opportunities will be provided. This is a multidisciplinary approach to oncology. Three years of internal medicine are required. *Please send curricula vitae and recommendations to:* Jules Lodish, MD, Director, Oncology Program, Mt. Sinai Medical Center, PO Box 342, Milwaukee, WI 53201.

Roswell Park Memorial Institute Continuing Education Seminars

Buffalo, New York, September 1980–June 1981

The Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on newest methods in the diagnosis and treatment of cancer. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee for 1-day seminars and a variable fee for longer programs. The seminars are co-sponsored by the Cancer Control Program of the Institute, and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Advances in Rehabilitation of the Cancer Patient, *September 18, 1980* (chairperson: Dr. Richard Berjian, Department of Rehabilitation); Trends in Oncology for the New Decade, *October 1–2, 1980* (chairperson: Dr. William Aungst, Associate Institute Director for Clinical Affairs); Advances in Head and Neck Oncology, *November 6, 1980* (chairperson: Dr. Donald Shedd, Department of Head and Neck surgery); Metastasis—Pathobiological Aspects with some Illustrative Clinical Examples, *December 4, 1980* (chairperson: Dr. Leonard Weiss, Department of Experimental Pathology); Advances in Gynecologic Oncology, *February 5, 1981* (chairperson: Dr. Joseph Barlow, Department of Gynecologic Oncology); Multimodal Treatment of Melanoma, *March 7, 1981* (chairperson: Dr. E. Douglas Holyoke, Department of Surgical Oncology); Management of Malignant Disease in Children, *April 2, 1981* (chairperson: Dr. Arnold Freeman, Department of Pediatrics); Hematologic Problems in Cancer Patients, *May 14, 1981* (chairperson: Dr. Julian L. Ambrus, Department of Pathophysiology); and Progress in the Management of Upper-Gastrointestinal Cancer, *June 6, 1981* (chairperson: Dr. Harold Douglass, Department of Surgical Oncology).

***Inquiries should be sent to:* Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St. Buffalo, NY 14263 (telephone: 716-845-4406).**

National Cancer Institute-European Organization for Research on Treatment of Cancer Symposium on "Nature, Prevention, and Treatment of Clinical Toxicity of Anticancer Agents"

Brussels, Belgium, September 25–27, 1980

The National Cancer Institute-European Organization for Research on Treatment of Cancer (NCI-EORTC) will present a symposium on "Nature, Prevention, and Treatment of Clinical Toxicity of Anticancer Agents" to be held at 2 rue Evers (1st floor), 1000 Brussels, Belgium, on September 25–27, 1980.

Invited lectures and free communications will be presented. *Abstracts for free communications* (in English, 250–300 words) *should be sent before August 1, 1980, to:* Dr. M. Staquet, EORTC Coordinator, Institut Jules Bordet, 1 rue Héger-Bordet, 1000 Brussels, Belgium. Phone: 538.57.90 – Telex 22773.

Attendance will be limited to the first 200 registrations. Early registration is advisable. The registration fee is 3000 Belgian francs. *To register please send your registration fee to:* EORTC Data Center, % Dr. M. Staquet, 1 rue Héger-Bordet, 1000 Brussels, Belgium.

Association of Professors of Medicine

Attention: **Directors of Subspecialty Fellowship Training Programs**

Call for revisions for the *Directory of Training Programs in Internal Medicine: Residency and Subspecialty Fellowships* (Washington, DC, Association of Professors of Medicine, 1979).

All subspecialty fellowship training programs in the US are listed in the above-referenced Directory. The Directory has been distributed to all directors of approved residency training programs in internal medicine and to all directors of subspecialty fellowship training programs. The Directory has been used by residents and others who wish information on the existence and location of the various programs.

To update the listing for 1980–1981 we need your help to identify the *beginning* of a new subspecialty fellowship training program, or the *amalgamation* of a program with another institution, or the *phasing out* of a program. If any of these changes has occurred in your training program, we ask that you please submit the requested information by October 15, 1980.

In order to be listed, the program must have at least *one* full-time Fellow in 1980–1981 whose *overall* direction is provided by the program director listed below. Please submit: the name of the program director, the subspecialty, the hospital, the address, and the phone number. Please indicate whether this program is (*a*) a new program; (*b*) amalgamating with another (if so, please indicate the name of the program director, the subspecialty, and the name and address of the *base* hospital); or (*c*) phasing out or terminating the training of fellows.

Please return to: **Alvin R. Tarlov, MD, Past President, Association of Professors of Medicine, One Dupont Circle, NW, Washington, DC 20036.**

Sixth Congress of the European Society for Medical Oncology

Hôtel Negresco, Nice, France, December 6–8, 1980

The Sixth Congress of the European Society for Medical Oncology will be held at the Hôtel Negresco, Nice, France, on December 6–8, 1980. Topics will include: diagnosis, treatment of cancer, and experimental studies. *For further information contact:* Prof. M. Schneider, Centre Antoine-Lacassagne, 36 Voie Romain, 06054 Nice Cedex, France.

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